Implementing Petri Nets for Modelling and Simulation in Biosciences

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ABSTRACT

It was in 1993 that Petri nets were introduced for modelling of biochemical reactions. Since that time Petri nets are increasingly used to dive deep into the details of functioning of cumbersome biological processes. In this context, Petri nets have become anindispensable tool for modeling and simulation of biochemical reactions, biomedical systems, processes arising in molecular biology and genetics.

This thesis intends to present a comprehensive overview of bibliography on application of Petri nets in biosciences. Based on analysis of bibliographical information covering the period of from January 1993 to July 2010, we provide statistical data on subject areas investigated with Petri nets, use of Petri net types, analysis methods as well as dedicated software tools.

We detail HFPN – a Petri net with extension that combines both discrete and continuous components in it – and Cell Illustrator 5.0 – a licensed powerful software tool for modeling and simulation of biological systems. In order to show effectiveness of both HFPN and Cell Illustrator in modelling and simulation of biopathways, we introduce three case studies: (i) validation of the p53 transcriptional activity through modelling with HFPN and performing simulation in Cell Illustrator software; (ii) modelling of gene regulatory mechanism of the *lac* operon and glycolytic pathway; (iii) circadian rhythms in *Drosophila*.

Keywords: Petri Nets,metabolic pathways, signal transduction networks, gene regulatory pathways

ÖZ

Petri ağları biyokimyasal reaksiyonları modellemek için ilk defa 1993 yılında önerilmiştir. O zamandan bu yana Petri ağları giderek daha karmaşık biyolojik süreçlerin işleyişini ayrıntılı olarak irdelemek için kullanılır.Bu bağlamda, Petri ağları biyokimyasal reaksiyonlarda, biyomedikal sistemlerde, moleküler biyoloji ve genetikte ortaya çıkan süreçlerin modellerinin oluşturulması ve simülasyon yapılması için vazgeçilmez bir araç haline gelmiştir.

Bu tezin amacı Petri ağlarının biyolojik bilimlerde uygulamalarına kapsamlı bir genel bakış sunmaktır.Bu amaçla Ocak 1993 - Temmuz 2010 dönemini kapsayan bibliyografik bilgilereistinaden, Petri ağları aracılığı ile araştırmalar yapılan biyolojik bilim alanlarına, Petri ağı türlerinin kullanımına, çözümleme yöntemlerineve özel yazılım araçlarına ilişkin istatistiki veriler sağlamiştır.

Hibrid fonksiyonel Petri ağı ayrık ve sürekli bileşenleri içinde birleştiren bir uzantıya sahip Petri ağı türüdür.Cell Illustrator 5.0 biyolojik süreçlerin modellenmesi ve simülasyonu için kullanılan etkili bir araçtır.Hem hibrid fonksiyonel Petri ağının hem de Cell Illustrator yazılımının etkinliği aşağıdaki üç örnekle irdelenmektedir:

(i)p53 transkripsiyonel aktifliğinin simülasyon yaparak doğrulanması;

(ii) *lac*operon gen düzenleyici mekanizmasının ve glikolitik yolun modelinin oluşturulması;

(iii) *Drosophila*için sirkadiyen ritim modelinin oluşturulması.

Anahtar Kelimeler: Petri ağları, metabolik yollar, sinyal iletimi kaskadlar, gen düzenleyici yollar

Dedicated to My Family

and

Mustafa DEMİRKAN

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Fatma DOLMA

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LIST OF ABBREVIATIONS

b-galactosidase	**B**eta-**galactosidase**
CPN	**C**olored **P**etri **N**et
CP-net	**C**olored **P**etri net
CO2	**C**arbon dioxide
DNA	**D**eoxyribo**n**ucleic **A**cid
E. coli	**E**scherichia **coli**
H2O	Water
HFPN	**H**ybrid **F**unctional **P**etri **N**et
HPN	**H**ybrid **P**etri **N**et
mRNA	**M**essenger **R**ibo**n**ucleic **A**cid
p53	**p**rotein **53**
P-invariant	**P**lace invariant
P/T-net	**P**lace **T**ransition **n**et
T-invariant	**T**ransition invariant

LIST OF SYMBOLS

A	the incidence matrix of a Petri net in the section (3.3.3)
A^T	the transpose of the matrix A
A	set of arcs in the section (3.1)
a_{ij}	the entries of the incidence matrix.
a_{ij}^-	the number of arcs connecting transition from place.
a_{ij}^+	the number of arcs connecting transition to place.
b	binding
B	set of bindings
c	color in the section (3.3.2)
c	name of the connector in the chapter(4and 5)
$C(p)$	set of colors of place
e	the name of entity
m	variable of the entity in the chapter 4 and 5
M_0	the initial marking in Petri net.
M_d	destination marking
p	place in the section (3.1)
P	set of places
p	the name of process in the chapter 4 and 5
t	transition
T	set of transitions
(p,t)	arc that is incident from place to transition
(t,p)	arc that is incident from transition to place

w	weight of arc
W	weight function
$\mathbf{x}$	firing count vector
$\Delta\mathbf{M}$	$\mathbf{M}_d - \mathbf{M}_0$

LIST OF TERMS

apoptosis	the process of programmed cell death that may occurin multicellular organisms
cell-to-cell communication	the process by which our cells communicate to each othother through the use of receptors
computational biology	the development and application of data-analytical andtheoretical methods, mathematical modeling and computational simulation techniques to the study of
Biochemistry	study of chemical processes in living organisms
bioengineering	a discipline that combines engineering expertisewithmedical needs for the enhancement in healthcare
bioinformatics	the use of information technology for the study,collection, and storage of genomic and other biologicaldata
biomathematics	the quantitative or mathematical study of biologicalprocesses, with an emphasis on modeling
biomedicine	branch of medical science that applies biological and other natural-science principles to clinical practice
biotechnology	controlled and deliberate manipulation of biologicalsystems (whether living cells or cell components) forthe efficient manufacture or processing of usefulproducts

Dimer	a complex consisting of two components
DNA	a nucleic acid that contains the genetic instructionsused in the development and functioning of all knownliving organisms and some viruses
embryogenesis	the process by which the embryo is formed and
Epistasis	the phenomenon where the effects of one gene are modified by one or several other genes, which are sometimes called modifier genes
gene	a molecular unit of heredity of a living organism
gene expression	the process by which information from a gene is usedin the synthesis of a functional gene product
in silico	performed on a computer or via computer simulation
in vitro	experiments done outside of living organisms
in vivo	experiments done in living organisms
MDM2	an important negative regulator of the p53 tumorsuppressor, which is used as the name of a gene aswell as the protein encoded by that gene
Membrane	typically defines enclosed space in a cells or compartments in which cells may maintain a chemicalor biochemicalenvironment that differs from the outside

metabolic disorder	a medical condition characterized by problemsconverting food to energy
metabolic pathway	a series of chemical reactions occurring within a cell
Metabolism	the set of chemical reactions that happen in the cells ofliving organisms to sustain life
molecular biology	the study of biology and biological functions atthe molecular level
p19ARF	a tumor suppressor that interacts with p53 and MDM2
p53	a tumor suppressor protein that prevents cellsfrom dividing inappropriately
Parkinson's disease	a degenerative disorder of the central nervous system
protein	biochemical compounds consisting of one or morepolypeptides typically folded into a globular or fibrousform, facilitating a biological function
signal transductionpathway	a set of chemical reactions in a cell that occurs when amolecule, such as a hormone, attaches to a receptor on the cell membrane
synthetic biology	a new area of biological research that combines science and engineering, that encompasses a variety of different approaches, methodologies, and disciplines with a variety of definitions, and which is aimed on the design and construction of new biological functions and systems not found in nature

systems biology	an emergent field that aims at system-levelunderstanding of biological systems
Transcription	the process of creating a complementaryRNA copy of a sequence of DNA
Translocation	the process of transportation of plants, animals orhabitat fromone location to another
trimer	a complex consisting of three components
Ubiquitination	a post-translational modification carried out by a set of three enzymes, E1, E2 and E3

Chapter 1

INTRODUCTION

Ordinary differential equations have been used over the years for modelling of biological processes. Genetic expression, genetic regulation, signal transduction and cellular reproduction exhibit a stochastic behavior. Modelling in terms of stochastic equations is another approach that is used to understand the nature of the processes in molecular biology. Implementing Bayesian networks, Boolean networks, state machines, generalized logic formalism, partial differential equations, qualitative differential equations and many other mathematical approaches in biosciences gave rise to an interdisciplinary field, which is known as mathematical biology or biomathematics for short. Biotechnology and bioengineering are among other interdisciplinary fields that are tightly coupled with biological sciences.

Over the past several decades, tremendous progress has been made in bioinformatics – another interdisciplinary field that brings together genomic and other biological data with the analytical methods of mathematics and software tools of computer science. Research in bioinformatics is aimed at deeper understanding of casual and functional relationship that generates dynamics of biological networks and pathways through use of computerized methods and techniques.

Petri nets concept was proposed in 1962 by Carl Adam Petri as mathematical formalism to enrich and expand automata theory has been successfully implemented

for modeling, analysis and simulation in engineering, scientific and industrial domains. There is nowadays tremendous interest in Petri nets regarding biosciences. To the best of our knowledge, it was in 1993 when Reddy et al. developed a Petri netmodel of the biochemical networks. Since that time, just a few papers appeared every year with similar approaches. There seems to be an increasing interest in that research topic, at least as far as we can tell from the total number of published papers.

In July 2010, we have gathered information from fourteen full-text data bases, which are legally accessible from Eastern Mediterranean University, and prepared critical overview of the Petri net based research methods, techniques and tools that have been implemented in biosciences. Our bibliography supplies 103 entries in biochemistry, 10 entries in biomedicine and 30 entries in molecular biology and systems biology. At first glance, it was observed that aforesaid research inspire new alliances between Petri nets and biosciences, to the benefit of both fields.

This work is a critical review of the bibliography on application of Petri nets in biosciences, which offers thorough and constructive insights into the existing studies in this field and casts the ongoing research in general setting. We propose a conceptual framework and discuss the role of Petri net based modeling, analysis and simulation approaches in three major areas: biochemistry, molecular and systems biology, and biomedicine. We report on variety of Petri net types and software tools which have been successfully used in biosciences. The outcome of this thesis is twofold: (1) revealing the variety of Petri net extensions and related software tools that can be involved for quantitative study of biological processes, with an emphasis on modeling; (2) diving deep into details of biological processes *in silico*.

Chapter 2is a systematical study of related works. We provide critical review of the bibliography on application of Petri nets in biosciences. Chapter 3 deals with Petri nets.Chapter 4 implements modelling and simulation software, Cell Illustrator with the HFPN of biopathway modeling and simulation. In Chapter 5, we provide three case studies. Firstly, we use Cell Illustrator software to create HFPN model of interactions between genes p53, MDM2 and p19ARFand perform simulations to study transcriptional activity of p53. Then we exploit HFPN and Cell Illustrator software for modelling the regulatory mechanism for *lac* operon gene and glycolyticpathway in *E. coli*. Finally, we create HFPN model of circadian rhythm of *Drosophila*. The conclusions are outlined at the end of the thesis.

Chapter 2

SYSTEMATICAL STUDY OF RELATED WORKS

In this chapter we (a) provide taxonomy of subject areas where Petri nets are extensively used for modelling, analysis and simulation; (b) discuss diversity in the implementation of Petri net types; (c) share observations regarding use of Petri net analysis methods; (d) give information on software tools.

2.1 Domain taxonomy

In the period of 1-31 July 2010, we have done research on implementation of Petri nets in biosciences. We have gathered information from thirteen full-text databases (see Table 2.1) that are legally accessible from Eastern Mediterranean University library.We have used Google as an internet search machine whenever it was necessary. Finally, we have chosen English as search language and restricted search tips with the following logical expression

(petri& !boite & !dish* & !plate*, "petri net*") & (biochemi*, biolog*, chemi*, ecolog*, medic*, metabol*, molecul*, react*, "signal trasduc*")

Table 2.1 List of full-text databases

Source name	**Link to the database**
Elsevier Science Direct	http://www.sciencedirect.com/
IEEE Xplore Database	http://www.ieeexplore.ieee.org/Xplore/dynhome.jsp?tag=1
Oxford Journals	http://www.oxfordjournals.org/
Springer link	http://www.springerlink.com/home/main.mpx
SIAM Journals Online	http://epubs.siam.org/
Wiley Interscience	http://www3.interscience.wiley.com/search/allsearch

Academic Search Complete	http://web.ebscohost.com/ehost/search?vid=1&hid=10&sid=c98a54d1-069d-4aa9-af52-e2ef4b8c5064%40sessionmgr13
ASME Digital Library	http://asmedl.aip.org/
Cambridge Journals	http://www.informatik.uni-hamburg.de/TGI/PetriNets/
ProQuest:Dissertations& Theses Full Text	http://proquest.umi.com/login
Computers & Applied Sciences Complete	http://web.ebscohost.com/ehost/search?vid=1&hid=9&sid=209bfacc-f586-4a30-8780-5a8085a16395%40sessionmgr4
Petri net bibliography	http://www.informatik.uni-hamburg.de/TGI/PetriNets/bibliographies/
CiteSeer	http://citeseer.nj.nec.com/cs

Distribution of the papers relative to the years is shown in Figure 2.1. A pioneering work in this field was published by Reddy [109] in 1993. Since 1993 until 2000, few papers published every year.

The number of papers published since 2000 is clear indication of growing interest in this research topic. Only 14 papers have been published up to end of July of 2010. It is expect that this number will be at least doubled by end of 2010. We observed that starting 2000 there is an increase in the number of papers published in this subject area.

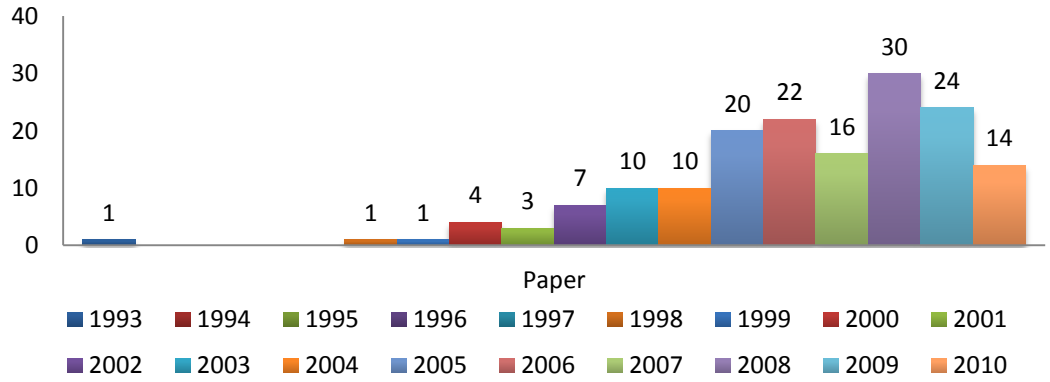


Figure 2.1 Number of published papers relative to years.

We have prepared a comprehensive review that is based on analysis of almost 150 journal papers, articles presented in symposia and published in proceedings,

technical reports, books, theses, etc. We have classified the subject areas into three classes: biochemistry, biomedicine, and molecular and systems biology. The distribution of the papers among three classes is as follows: 103 papers in biochemistry, 10 papers in biomedicine, and 30 papers in molecular and systems biology.

Biochemistry, which studies the chemical reactions required for life to exist, is the largest area where Petri nets have been effectively applied. The three subclasses within biochemistry that heavily attract experts are gene (or genetic) regulatory networks, metabolic pathways and signal transduction pathways (or signaling networks). A common name for above three subclasses is biochemical networks. These subclasses include 26, 27 and 29 papers, respectively. We had difficulty with determining subclass for 14 papers though all those papers perfectly fit in biochemistry.

Molecular biology, whichstudiesliving organisms and their biological functions at the molecular level,and systems biology, which puts together biology and system theory to develop a systemized learning environment for problems of biological nature, represent another large class that is rich of Petri net applications. We found that the contents in 20 papers fully agree with systems biology or computational systems biology. 10 papers are fall in molecular biology. We have found that 10 papers are dedicated to use of Petri nets in biomedical research. Figure 2.2 shows taxonomy of areas with Petri nets have been implemented for modelling and simulation.

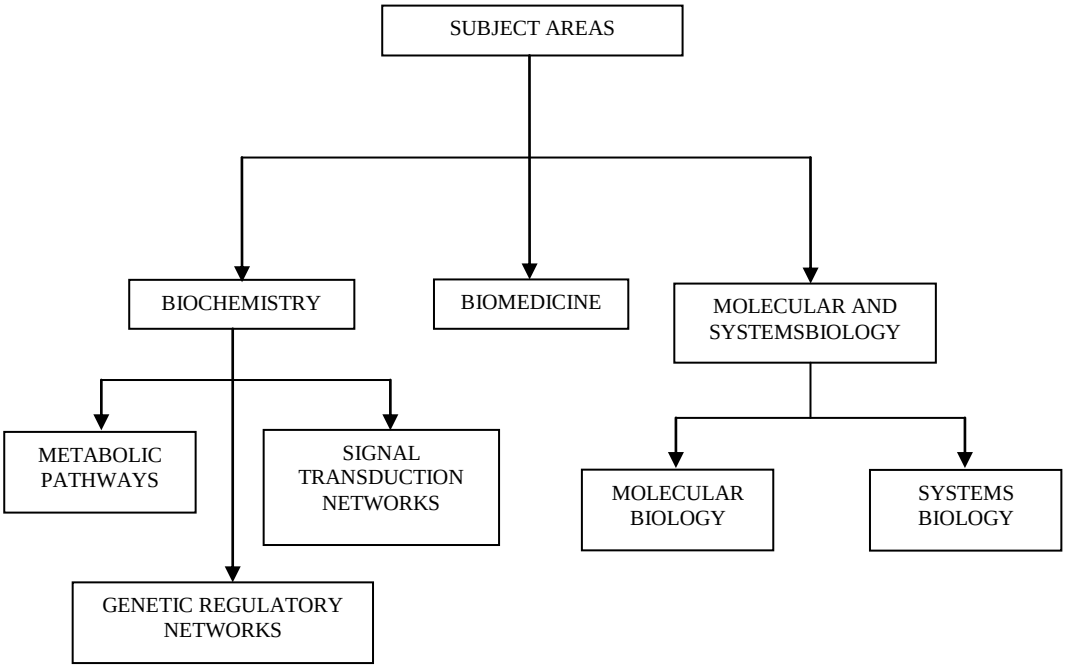


Figure. 2.2 Taxonomy of subject areas with Petri net applications.

Table 2.2 provides more detailed information on topics being considered for modelling, analysis and simulation with Petri nets. In this table we indicate the number of citation and references for each topic.

Table 2.2 References to papers considering Petri netsfor biosciences.

Subject	**References**
Apoptosis	[51]
Amphibian metamorphosis	[130]
Biochemical networks	[2,10,37,44,54,55,57,66,73,80]
Biochemical systems	[10,82,100,101,138,139,140]
Biochemistry	[87,138]
Bioinformatics	[22,57,82,118]
Biological pathways	[32,51,60,71,83,92,93,133,141]
Biological network (or system)	[11,15,37,43,44,48,66,78,80,81,99,104,126]
Boolean networks	[5]
Cellular rhythm	[55,60]
Cell-to-cell communication	[55,59]
Chemical reaction network	[2]
Computational systems biology	[25,81,87,96,107]
DNA computing	[118]
Embryogenesis	[87]
Epidemiological model	[4]
Epistasis	[85,89]

Flower development (or morphogenesis)	[53,63,122]
Gene-gene interaction	[85,89]
Genetic regulatory networks	[5,35,37,41,42,53,61,63,65,80,88,96,110,125]
Gene expression	[15,43,93,126]
Iron homeostasis (or metabolism)	[9, 113, 114]
Medical bioinformatics (or biomedicine)	[22]
Membrane system	[16,67]
Metabolic disorders	[22]
Metabolic pathway (or network)	[29, 43, 69, 73, 83, 105, 107, 114, 118]
Metabolism	[3,121,123,145]
Metal metabolism	[27]
Modelling molecular interactions	[8]
Molecular networks	[35]
Parkinson's disease	[140]
Protein production (or transport)	[6,122]
Signal transduction network (or pathway)	[10,12,35,51,55,75,83,115,129,142]
Signal transition graphs	[5]
Signalling pathway	[10,54,75]
Synthetic biology	[16,34,57]
Systems biology	[16,22,25,38,50,75,78,92,96,119]

2.2 Research Methods and Research Skills

Over the years, Petri net formalism has been expanded to support characteristics of the problems arising in various areas. As we believe, there should be a reasonable trade-off between expressiveness of the formalism and simplicity of its implementation. The more expressive the formalism, the more difficult the analysis becomes.

Statistical data regarding use of Petri net types is provided in Figure. 2.3. It can be easily observed that P/T-net is the most frequently referred Petri net type. P/T-net has been referred in 52 papers or in almost every third paper. Simplicity of modelling, easiness in implementation and richness of analysis methods makes P/T-net indispensable tool for modelling and analysis of biological problems.

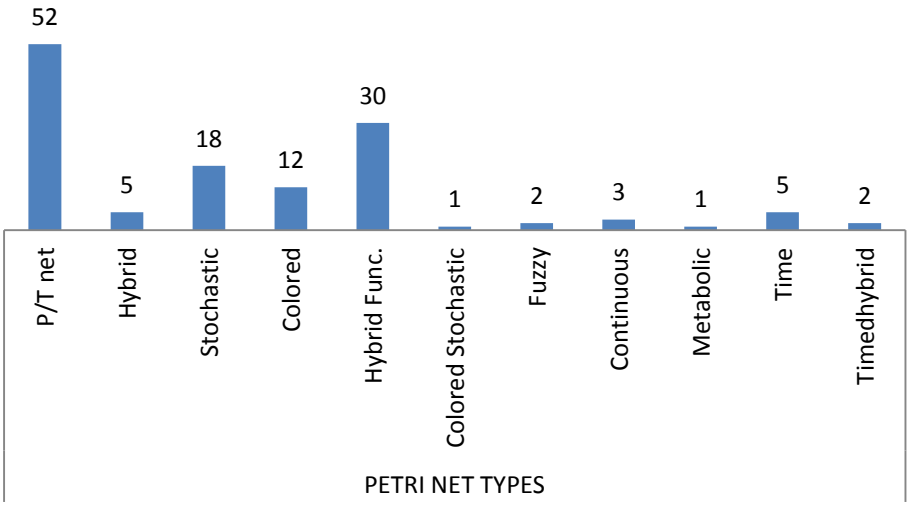


Figure 2.3Statistics on use of Petri net types.

HFPN is the second most preferred Petri net type. HFPNs have been used in almost every fifth paper. In addition, timed hybrid and hybrid Petri nets are referred in 2 and 5 papers, respectively. Altogether, hybrid Petri nets with or without additional extensions have been referred in 37 papers. Two attractive and powerful features make hybrid Petri nets powerful modelling tool: they are discrete and continuous at the same time. Stochastic and colored Petri nets with 18 and 12 references are among other preferred Petri net types.

In 30 papers Petri nets have been considered for modelling only, in 3 papers for simulation only, and finally in 84 papers for both modelling and simulation reasons. In majority of papers Petri nets are used for both modelling and simulation reasons. This is clear indication of what practitioners expect from Petri nets.

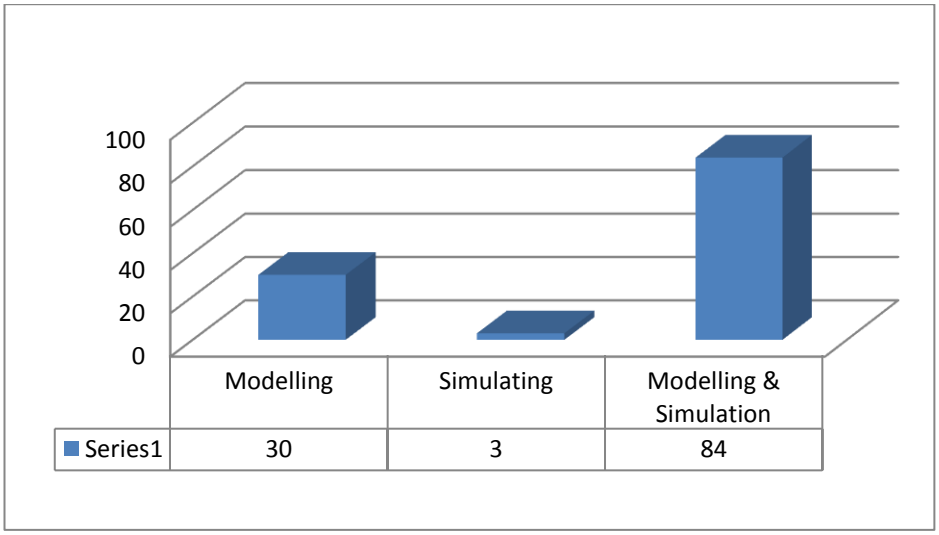


Figure 2.4Statistics on use of Petri nets.

Invariants, P-invariants and T-invariants methods are widely used for analysis of biological problems. In 89 papers, these methods are explicitly implemented for finding invariants and reachability analysis. It is somewhat surprising that other Petri net analysis methods have been not used at all. Statistics on use of Petri net analysis methods is shown in Figure 2.5.

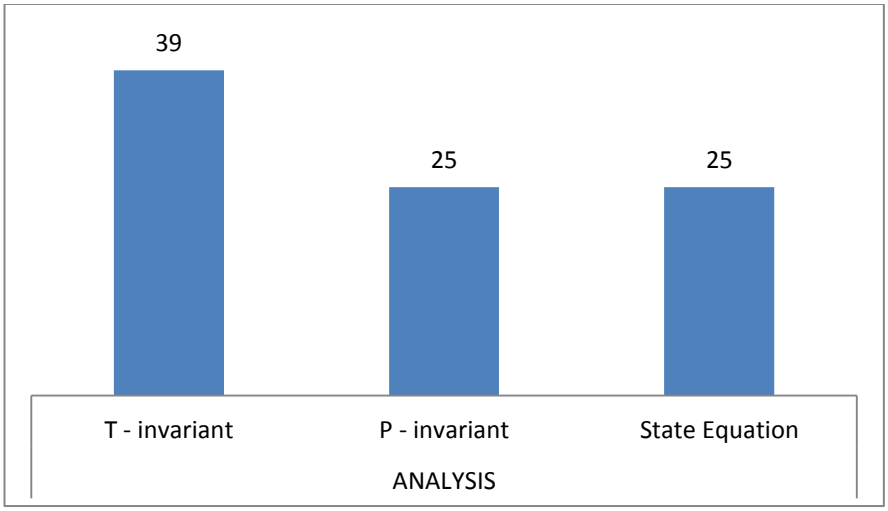


Figure 2.5Statistics on use of Petri net analysis methods.

2.3 Software Tools in Use

There are many software tools for Petri nets. Selection of appropriate software tool depends on several parameters such as extension of Petri net, analysis methods to be used, and characteristics of the problem. Quick overview of software tools for Petri Nets and statistics on use of these software tools are provided in Table 2.3.

Table 2.3List of software tools for Petri nets and frequency of the use.

Software tool	**Number of papers**
Akt and MAPK pathways	2
AutomaticNetworkReconstruction	1
BIOCHAM	3
BioMedols	2
CADLIVE	1
CADP	1
CaMKII	2
CELISCA	1
Cell Illustrator	10
Cell System Ontology	2
CellDesigner	2
Cellular automata	1
Charlie	1
Daikon	1
DBToolKit	1
DiVinE	1
E-CELL	4
FPN	2
GALLOPS	2
GALS	1
Gene Ontology	1
GeneChip	1
GEPASI	4
GINsim	5
GNA	1
GNaPN	1
GeneticNetworkAnalyser	1
JigCell	1
KEGG database	7
MetaCyc	1
MetaPlab	1
metaSHARK	1
METATOOL	5
MissRdP	1
MODELICA	1
ModelMaker	1
MoVisPP	1
NUSMV	1
Pathway Logic	1
PATIKA	1
PEP	1

PNML	1
PRANSPATH database	2
PTRL-net	1
SARGE	1
SBML	2
SEPARATOR	1
SimBiology	1
SimCell	1
Toolkit107	1
TPO signaling pathway	1
ULTRASAN	1
Woflan	1

Cell Illustrator software tool, which has been designed in Miyano Laboratory at University of Tokyo, is the most frequently used software tool. Cell Illustrator is used in 10 papers. Capability to support both discrete and continuous events is among other attractive features of Cell Illustrator. KEGG database represents a biological database with dedicated software tool supporting it. Having reference number 7, KEGG database is the next highly referred software tool. METATOOL and GINSim are used in 5 papers each. It should be noticed that most of the software tools are originally designed to support biological nature of the problems.

Chapter 3

PETRI NETS

History of Petri nets began in 1962 with a thesis of Carl Adam Petri. Since then, hundreds if not thousands papers have been published in this field. Over the years essential progress has been made in theoretical research done in Petri nets aiming at expanding the formalism behind of the Petri nets and widening implementation areas of Petri nets.

Petri nets theory is a successor of theory of automata enriching this field by new theoretical results. On the other hand, Petri nets is a practical tool, that is suitable for modelling, analysis and simulation of dynamic systems. Petri nets have been applied for problem solving in wide spectrum of engineering, scientific and industrial areas. Concurrent systems parallel and distributed computing, performance evaluation, computer architecture, asynchronous systems, computer networks, operating systems, fault-tolerance systems, distributed database systems, dataflow computing systems, discrete event industrial systems are among other fields where Petri nets have successfully been implemented for problem modelling and simulation. There is nowadays tremendous interest in use of Petri nets in biochemistry, biomedicine, molecular and systems biology.

3.1 Background

Petri net can be formally defined as 5-tuple $PT =< P, T, A, W, M_0 >$ where$P = p_1, \dots, p_m$ is the set of places, $T = t_1, \dots, t_n$ is the set of transitions,$A \subseteq$

$T \times P \cup P \times T$ is the set of arcs, $W: A \rightarrow 1,2,3,\ldots$ is the weight function, $M_0: P \rightarrow 1,2,3,\ldots$ is the initial function, and $P \cap T = \emptyset$.

A Petri net consists of set of places P, set of transitions T, and set of arcs A. An arc is incident from a place to transition or vice versa. A place p is input place of transition t if and only if $(p, t) \in A$. Likewise, place p is output place of transition t if and only if $(t, p) \in A$. It should also be noticed that arcs connect objects of different types but not the same type. In applications, input places are used to model preconditions, input data, input signals, resources needed, conditions, buffer. Output places are interpreted as postconditions, output data, output signals, resources released, conclusions, buffer. Actions like events, computation step, signal processor, task for job, clause in logic and processor are modeled by transitions. Weights function is used to identify the multiplicity of the arcs. Weight function $w(p, t)$ (or $w\ t, p\ $) is used to indicate the number of arcs between (p, t) (or (t, p)).

It is customary to characterize the behavior of dynamic systems by their states. In order to understand system's behavior, it is important to investigate set of states and possible transitions between the states. In a Petri net model, system state is specified by marking. A marking can be thought of as collection of tokens distributed among the places. In the beginning, a system is set to the initial marking M_0. Any change in the system's state changes settlement of the tokens in the Petri net consequently moving Petri net to a new marking.

Structural analysis of Petri nets is separate topic of research. Behavioral analysis seems to be more important from practical implementation perspective.

A transition is said to be enable if each input place contains at least as much tokens as the weight of the corresponding arc. Otherwise it is disable. Enable transition may occur (or fire). Occurrence of a transition changes state of the Petri net.

Example 1: Shows transition firing rule process:

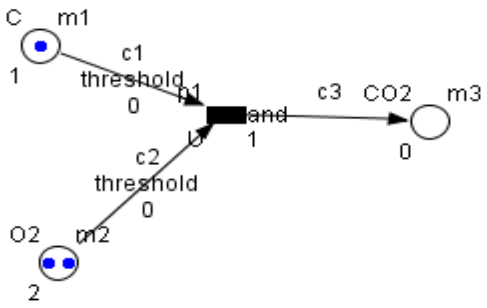


Figure3.1: Transition firing rule for marking before firing the enabled transition

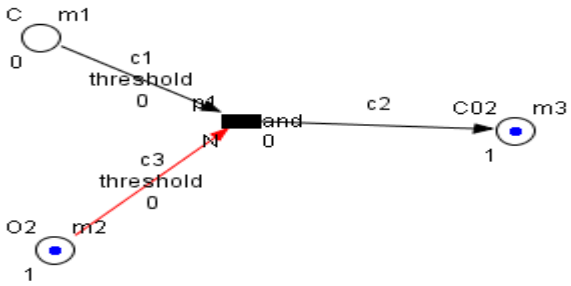


Figure3.2: Transition firing rule for marking after firing p1, whereis p1 disabled.

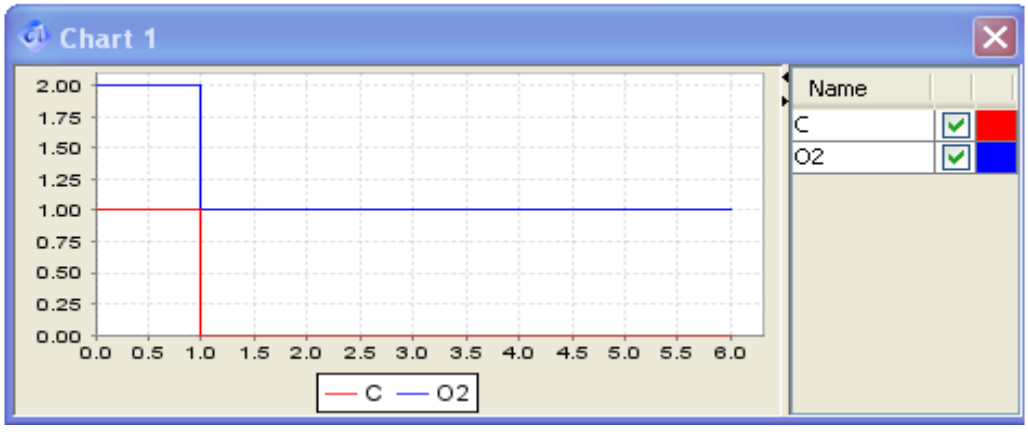


Figure3.3: Shows the simulation of example

3.2 Petri Nets with extensions

In general, we distinguish between two classes of Petri nets:P/T-nets and high-level Petri nets. P/T-nets, that are also called ordinary Petri nets, are easy in use and many problems can be modeled and analyzed in terms of P/T-nets. Unfortunately, P/T-nets turn out to be too simple to describe complex specifications of dynamic systems. To overcome this problem the definition of classical Petri net can be expanded with new characteristics. Timed Petri nets, CP-nets, stochastic Petri nets, fuzzy Petri netsand hierarchical Petri nets are all high-level Petri nets. It is quite often that a combination of multiple Petri net types is used to create adequate model of the systems. Timed CP-net, hierarchical CP-net, timed stochastic Petri net, timed hierarchical CP-net are just few examples to high level Petri nets employing multiple characteristics.

3.2.1 CP-nets

CP-nets represent interesting subclass of high-level Petri nets.In a CP-net tokens are attached data values of specified type called colors, so that each token is associated with attributes. These attributes are calledcolors. Most the rules of a program coding are valid for CP-nets as well. Occurrence of a transition initializes variables to corresponding values. There must be agreement between color set, variable types and assigned values. CP-nets have been successfully used in telephony, communications protocols, resource allocation, operation systems and some other areas.

3.2.2 Hierarchical Petri nets

It is rather difficult to create large complicated Petri nets. Instead of creating a large Petri net one would prefer to split the model into submodels of reasonable size and keep each submodel on a separate level. Hierarchical CP-nets are nets are multilayer nets. There exist two trends in design hierarchical Petri nets: top-to-down and down-to-top design styles. In parallel with the modular programming, you can create

simple net that gives a broad picture of the system. By substituting the object at the toplevelnet with more detailed subnets, you can get complete picture of the model. Modelling with hierarchical Petri nets is efficient if you are capable to distribute a model across multiple levels, to divide it into modules small enough to keep track.

3.2.3 Petri nets with time extension

Petri nets with time extension present another interesting type of Petri nets. Practitioners compromise between timed Petri net and time Petri net. Although these two Petri net extensions are seemed to be perfectly same there is a difference between them. In a timed Petri net a transition fires as soon as possible, whereas in a time Petri net it fires within a time period. In a timed Petri net time parameter can be considered relative places or transitions.

3.2.4 Hybrid Petri nets

The places and transitions in a HPNare either discrete or continuous. Definition and use of a discrete place and a discrete transition are same to those in classical Petri nets. A continuous place holds a nonnegative real number. Real number attached to the continuous place decreases or increases at predefined speed with each occurrenceof a continuous transition. An arc is either normal, inhibitory or test arc. HPN's components in all possible shapes are illustrated in Figure 3.4.

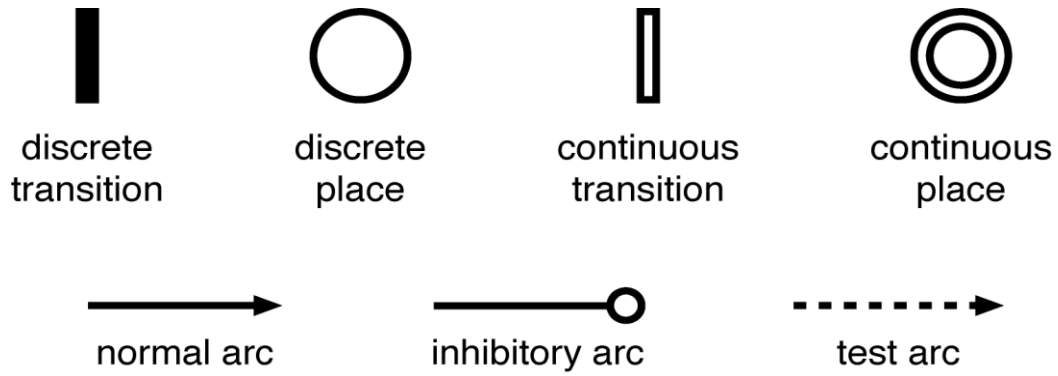


Figure3.4 Shapes used for discrete and continuous components in HPNs.

A normal arc is between a pair of discrete/continuous transition and place. An inhibitory arc havingweight *w* enables the transition if and only if the content of the place at the source of the arc does not exceed *w*. For instance, an inhibitory arc can represent a repressive activity in gene regulation. A *test arc* does not change the content of the input place.

3.3 Analysis Methods

Analysis with Petri nets falls into three classes: the reachability or coverability tree (or graph) methods, the matrix equation methods, and reduction or decomposition methods. The reachability tree method is based on exhaustive search for all possible markings that are reachable from specified marking. Due to complexity problems, which often causes memory overflow called "state space explosion", use of reachability tree method is limited to modest-size Petri nets. Matrix equation and reduction or decomposition methods are applied to a limited subset of places rather than whole set of places and consequently they are more powerful. The only drawback of matrix equation and reduction or decomposition methods is that they are applied to Petri nets of special types.

3.3.1 Reachability and coverability tree methods

Drawing reachability tree, however, may grow infinitely if the Petri net is unbounded. To keep the tree as compact as possible, we use the method of coverability tree. A coverability tree is obtained by folding of original reachability tree. We introduce the symbol ω which stands for "infinity". This symbol is used to indicate the number of the tokens in coverable markings. The operations allowed with ω are defined as $\omega > n$, $\omega \mp n = \omega$, and $\omega \geq \omega$ where n is an arbitrary integer. Drawing of a particular branch in a coverability tree terminates if one of the following cases is encountered: (i) the present marking is a repeated marking; (ii) the

present marking is a dead marking; (iii) the present marking is coverable marking. In the latter case we use the symbol ω and operations introduced for ω to indicate the number of the tokens in coverable places.

3.3.2 Reduction and decomposition methods

In order to facilitate the analysis of large and cumbersome models it is customary to reduce Petri nets into equivalent but simpler ones while preserving the systems characteristics. The main idea behind of reduction or decomposition is as follows. It is sometimes hard to check a Petri net for satiability of certain properties. This may be because of extension that is attached to the Petri net. This unwanted situation can be avoided by converting original abstract Petri net into equivalent but less abstract Petri net that allows us to check for desired properties. Below we detail this idea on example of unfolding CP-net into equivalent P/T-net.

In a CP-net token are attached complex data types. Because of complicated token syntax, it is difficult if not impossible to use the method of P-invariants or T-invariants for reachability analysis in a CP-net. If this is the case then unfolding technique can be used to transform corresponding CP-net into equivalent P/T-net preserving the main properties of the original net. Unfolding is obtained by successive implementation of the following steps:

1. Unfold each colored place $p \in P$ into a set of places $p' \in P'$ one for each color of tokens $c \in C(p)$.
2. Unfold each colored transition $t \in T$ into a set of transitions $t' \in T'$, one for each binding $b \in B\ t$.
3. Represent an unfolded net in a compact symbolic form by utilizing Data Decision Diagrams.

4. Optimize an unfolded net by removing all unnecessary components such as 0-bounded places, transitions with false-valued guards, etc.

3.3.3 The method of matrix equation

For a PT-net with m places and n trasitions, an incidence matrix $A = [a_{ij}]$ is defined to be $n \times m$ matrix with the elements $a_{ij} = a_{ij}^{-} - a_{ij}^{+}$ such that $a_{ij}^{+} = w(i,j)$ is the weight of arc from transition i to place j and $a_{ij}^{-} = w(j,i)$ is the weight of arc from place j to transition i. The components a_{ij}^{-}, a_{ij}^{+} anda_{ij} represent the number of tokens removed, added and changed in place j when transition i occurs. Firing count vector $x = (x_i)$ is $n \times 1$ column vector of nonnegative integers with x_i being the number of occurrences of transition i. The initial marking M_0 and destination marking M_d are $m \times 1$ column vectors indicating state of the system in the beginning and at the end. ΔMdenotes the number of changed tokens in places while state changes from M_0 to M_d.

The method of T-invariants analyzes reachability of marking M_d from M_0 in terms of matrix equation:

$$A^T \cdot x = \Delta M$$

whereA^T is the transposed matrix. In the method of P-invariants, aforesaid matrix equation is expressed in the form:

$$x^T \cdot A = \Delta M^T$$

wherex^T and ΔM^T are transposed vectors.

In general, M_d is reachable from M_0 if there exists a firing count vector x such that the related matrix equation has nonnegative solution. In the case of no solution, M_d is

not reachable from M_0. It has been proved that for general Petri nets existence of nonnegative firing count vector x is necessary but not sufficient condition. Thus, for general Petri net existence of nonnegative solution does not guarantee reachability of desired marking from the initial marking. For acyclic Petri nets, however, it is also sufficient condition for reachability.

Chapter 4

MODELLING AND SIMULATION

4.1Cell Illustrator

It's drawing capabilities is very excellent, is also used in metabolic pathways modeling, signal transduction cascades, gene regulatory pathways and also used dynamically in the interactions of some biological entities like genomic DNA, mRNA and proteins.

Cell illustrator is powerful software for examining biological pathways, for illustrating experimental data and trial assumptions. Cell Illustrator also provides all the data that is necessary to analyze the results of computer experiments including charts, figures, etc.

4.2 Elements of Cell Illustrator

The three types of elements of modeling in Petri nets which are places, transitions and arcs are a subsequently represented in cell illustrator as entities, processes and connectors respectively.

4.2.1 Definitions of Entities

Entities are abstract elements that can represent any type of biological concepts like mRNAs, DNA, proteins, ligands, and compounds. They can also represent cellular structures like mitochondria, cell nuclei, cells or biological phenomena like transcription and translation.

The entities are classified as discrete, continuous and generic entities.

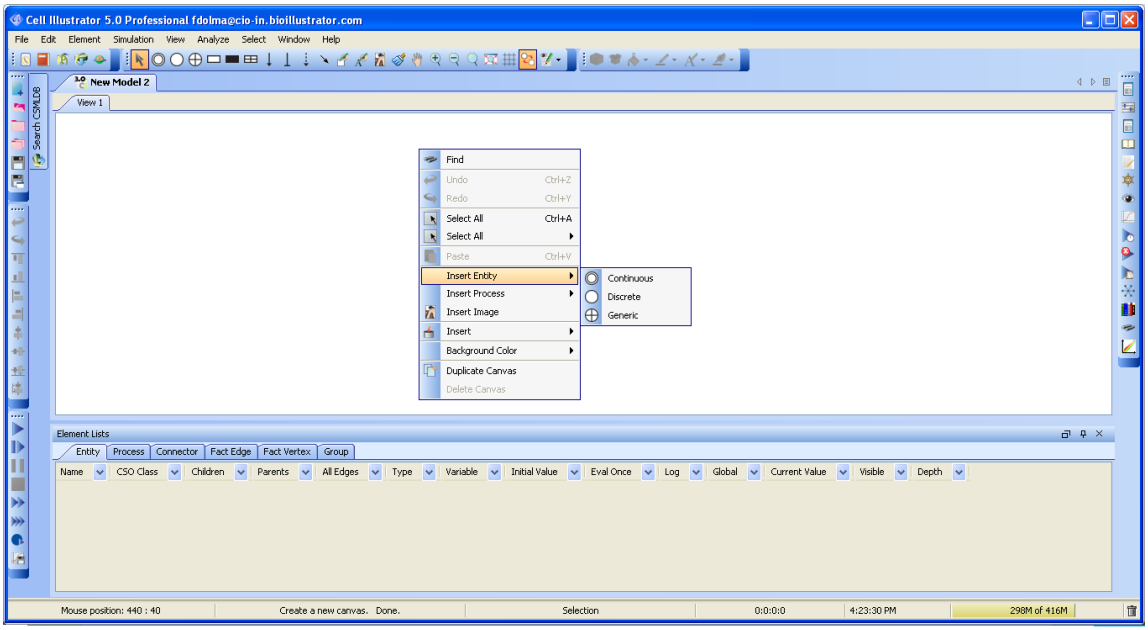
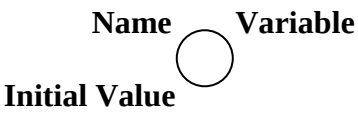


Figure 4.1: Shows the different forms of entities

Entities has three properties: Name, Variable and Initial Value



Definition: Differences between the entities

Discrete entity holds an integer quantity (Integer or Long). In contrast, a continuous entity has a continuous (e.g. real / fractional) number as its quantity (Double). This type of entity is used to represent concentrations, e.g. the number of ions or enzymes. Other type of entity is a generic entity. It holds string or logical value (String or Boolean).

4.2.2 Connectors

Connectors are used to link or connect entities and processes. Input connectors are connectors that connect entities to processes while output connectors link or connect processes to entities.

The three kinds of connectors (Figure4.2) are process connectors, inhibitory connectors and association connectors. Any of the connectors can be used as input connector while only process connector can be used as output connector.

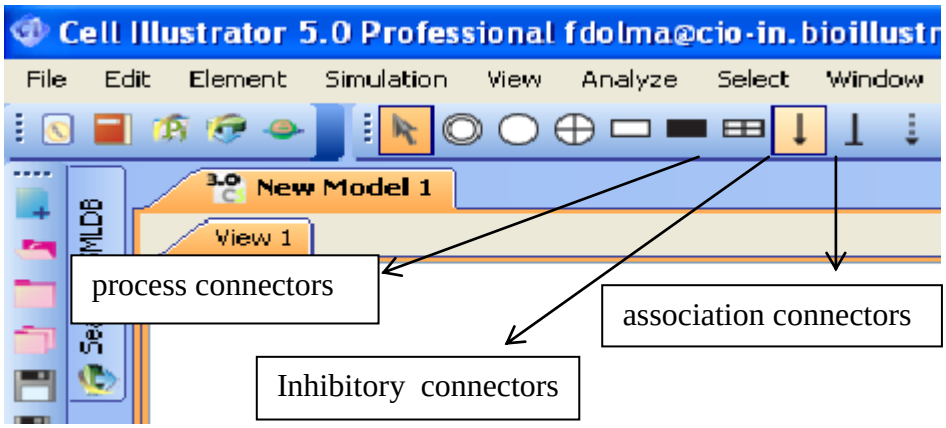


Figure 4.2: Shows the different kinds of connectors

Connector has two properties: Name & Threshold.

Name

Threshold

Only input connector's has threshold parameter that determines the activation or deactivation of the connecting process. The initial value of input entity should be greater than threshold of the connector in order to activate the process and an association connector. Contrarily, if the threshold is greater than the initial value of the input entity then activation occurs in inhibitory connectors. Repression modeling is achieved with the use of inhibitory connectors and when entities and process are to be connected together, the association connectors are preferably used for such modeling. This association connectors which disallow transport of quantities are used mostly input entities distributions is unchanged.

4.2.3 Definition of Processes

Processes (Figure 4.3)define the rate of entity value changes and interactionsamong entities. Processes are used to model biological reactions such as enzymaticreactions

or protein complex formation processes. Processes can take multiple and have multiple outputs.There are three process types: discrete, continuous and generic processes.

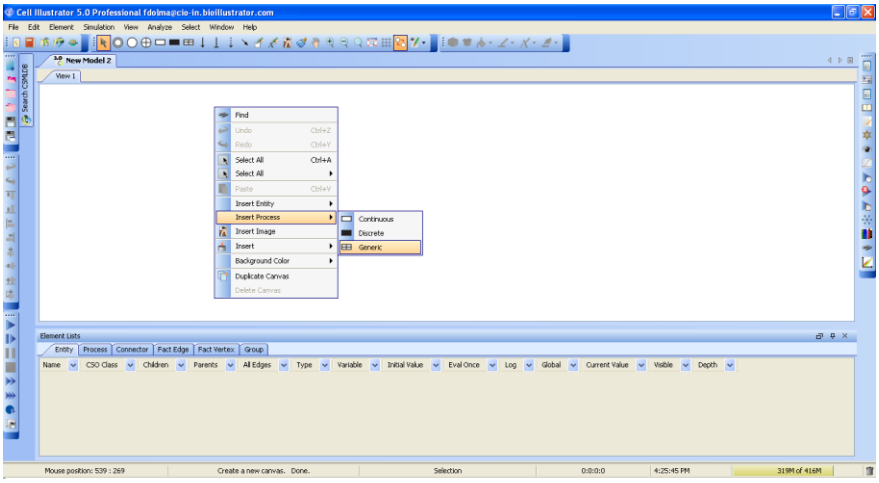


Figure 4.3: Processes

Process has three properties: Name, Delay and Speed.

Name

Delay Speed

Delay is used with discrete processes only.

To fire an enabled process it is necessary that all input connectors of a process to be activated. In addition, the reaction that follows is fired instantly or just in a short period.

Then, the value of all connected entities changes. Take for instance, processes that illustrate the reaction of a protein precursor that is converted into an activated protein by phosphorylation. Immediately the process fires, the speed of phosphorylation determines the simulation of the decrease of the precursor protein and subsequent increase in the active protein.

The firing style mainly and other process parameters are the necessary conditions to enable a process. The calculation style dictates the process type. A continuous process is with speed calculation style, which calculates the speed of production or consumption of the entities. Otherwise, a discrete process is with adding style, which calculates the concrete values required to be added or subtracted from the entities.

Lastly, for a generic process, its calculation style is update. This means that the entity value is replaced with a concrete value that the process calculates. How to calculate the change in value of connected entities in all three cases are particularize the Kinetic Script parameter.

4.3 Graphical Elements

4.3.1 The Biological Elements frame includes the Entity, Process and CellComponent tabs.

4.3.1.1 Entity

The Entity tab has biological elements that will include a particular quantity like H2O and CO2.

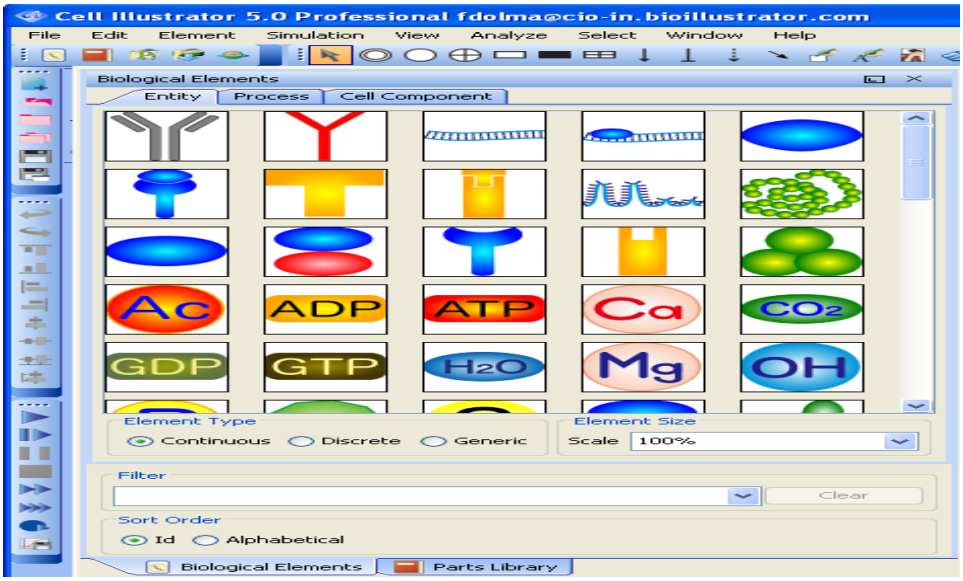


Figure 4.4: Biological entities

4.3.1.2 Process

The Process tabincludes biological elements that show biological processes like binding and degradation…etc.

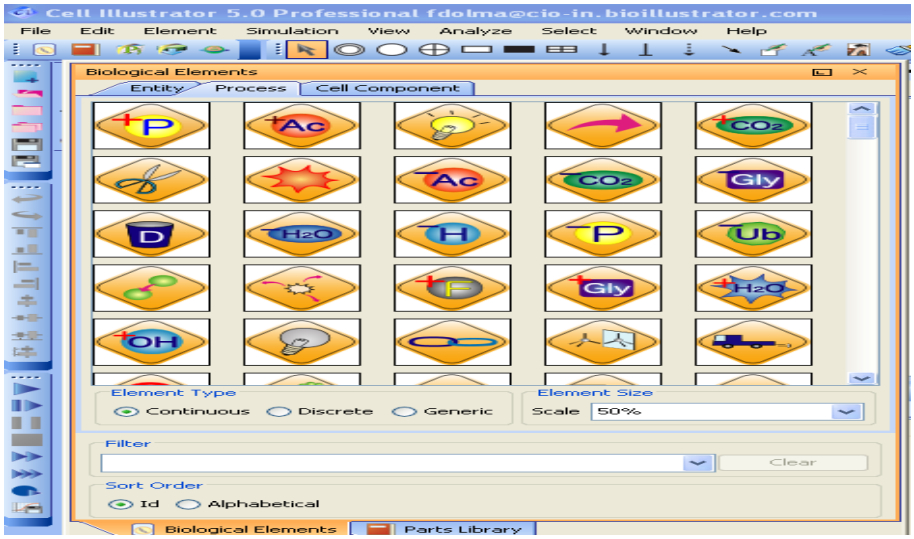


Figure 4.5: Biological processes

4.3.1.3 Cell Component

The Cell Component tab contains pictures of cellular components like centrosome and chloroplast… etc.

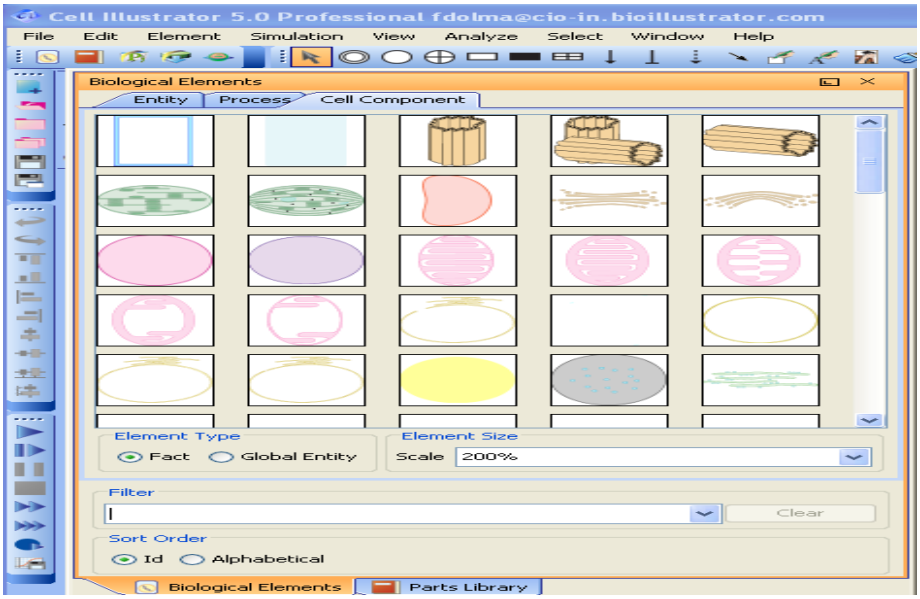


Figure 4.6: Cell components

4.4RULES OF SIMULATION

It is easy to perform simulation, which connects entity and process. In this section, we are going to explain that how can we do complicate model after adding elements properties to an easy model.

Example 1: Discrete Entities and Discrete process

Figure 4.7 is shown that initial values of all entities are zero thus initial values do not change during simulation as shown in Figure 4.8.

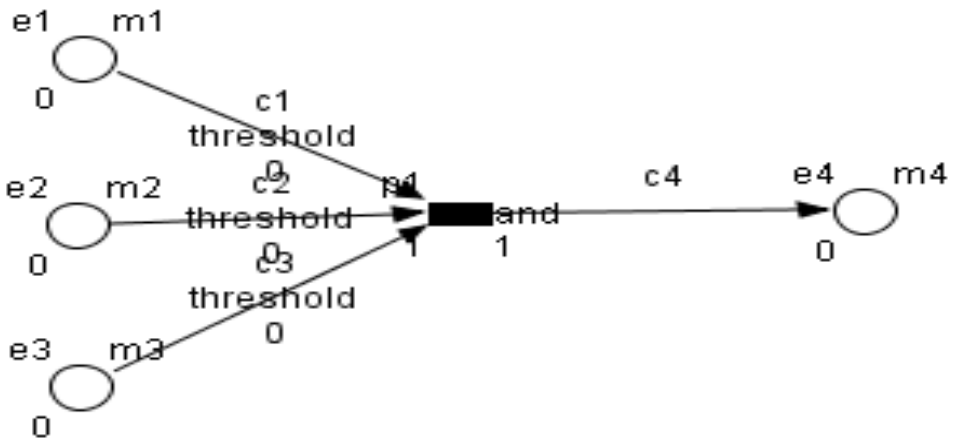


Figure 4.7: Hybrid functional Petri net model of discrete elements.

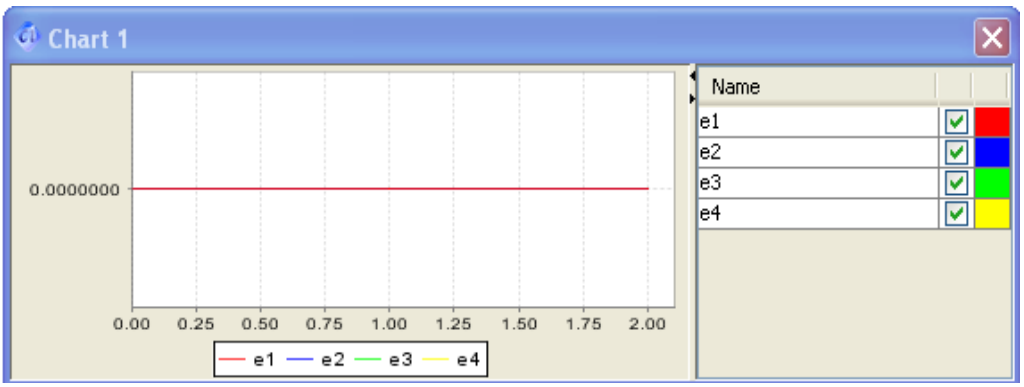


Figure 4.8: Simulation of discrete entities and discrete process.

4.4.1 Initial Value

If we change initial values of e1, e2 and e3 to 8 like in Figure 4.9, simulation produces chart at Figure 4.10.Process stops after values of e1, e2 and e3 become zero and it does not change again value of entity becomes zero.

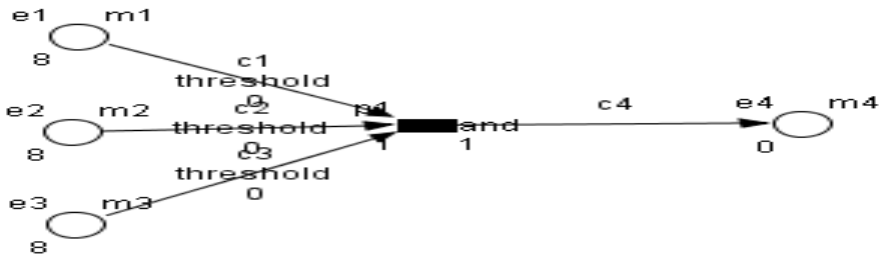


Figure 4.9 Modelling of discrete elements for change initial values.

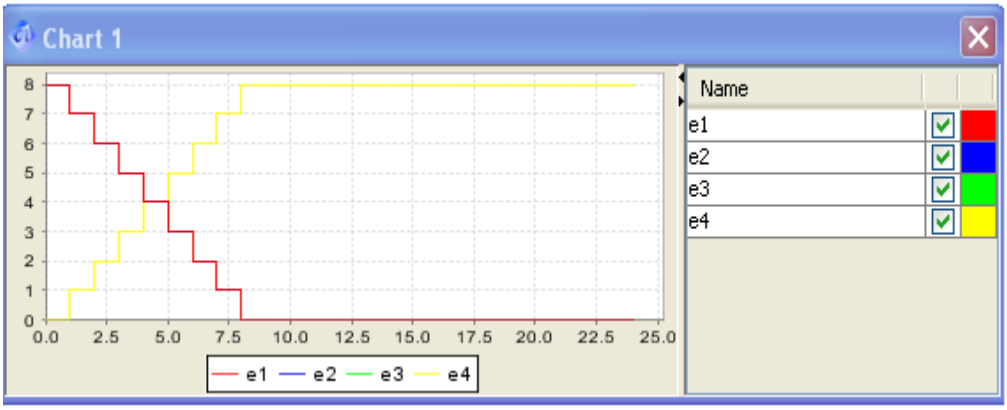


Figure4.10: Simulationof discrete elements for change initial values.

4.4.2 Speed

The values of the places e1, e2 and e3 get changed from 8[pt] to 4[pt] when speed is changed from 1 to 2, meaning that whole process takes twice less time when we increase the action speed. This is illustrated in Figure 4.12. The speed of the process is always regulated by speed parameter.

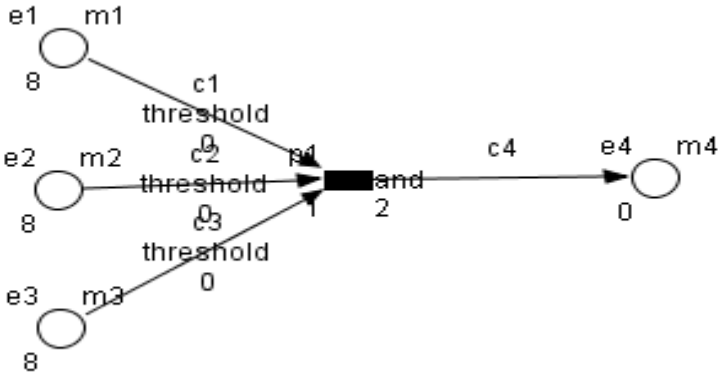


Figure 4.11 Modelling of discrete elements for change speed.

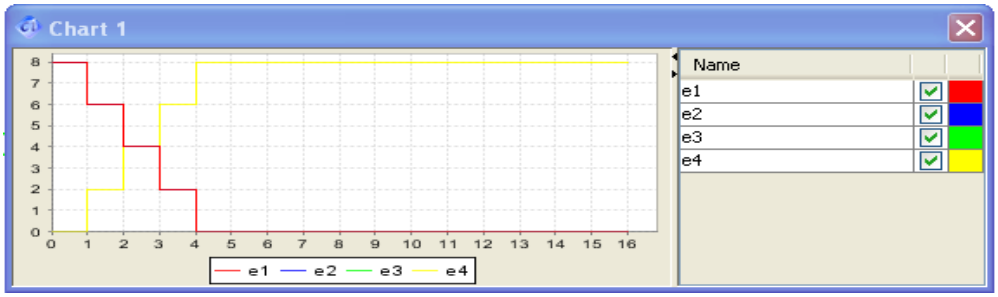


Figure 4.12: Simulationof discrete elements for change speed.

4.4.3 Delay

The delay parameter controls the process suspension time.Let us now change the delay parameter for p1 from 1 to 2. The simulation results are showninFigure4.14. It must be mentioned that simulation for model that is described in Figure4.9gets to completion at time 8[pt]. The values however are changed at every 1[pt] (see Figure 4.10) and 2[pt] (see Figure 4.14).Interval between the differences in values of the particular process can be controlled by relationship between speed parameter and delay parameter.

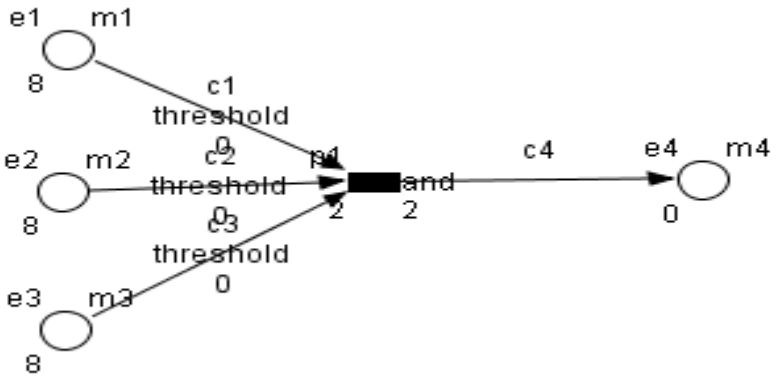


Figure4.13 Modelling of discrete elements for change delay.

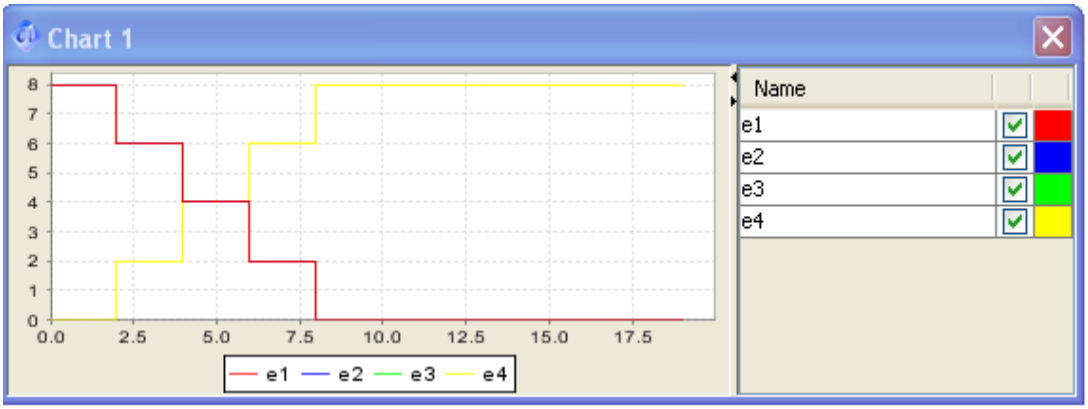


Figure4.14 Simulationof discrete elements for change delay.

4.4.4 Threshold

When we change threshold to 4 then run simulation, we obtain Figure 4.16. Values of e1, e2 and e3 have not been changed after 4[pt] time. When an input entity is smaller than the threshold of the connector, the value of the input entity is not be moved to the target process. In this example, for c2 and c3 threshold is suitable at time 4[pt] because threshold is 0. Therefore, c1 is not suitable for why threshold of c1 is equals to 4 and this value is equals values of c1, c2 and c3 so process has stopped.

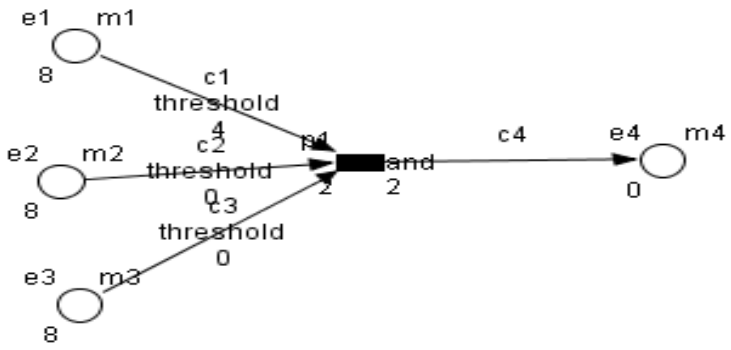


Figure 4.15 Modeling of discrete elements for change threshold.

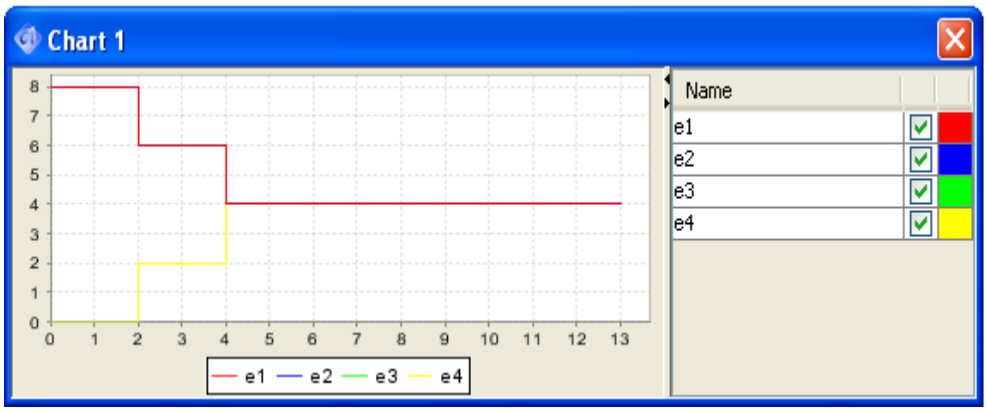


Figure 4.16 Simulationof discrete elements for change threshold.

Example 2: Continuous Entities and Continuous process

Initials values of all entities are 0 like discrete model where is in Figure 4.17 thence initial values has not been changed during simulation like shown in Figure 4.18.

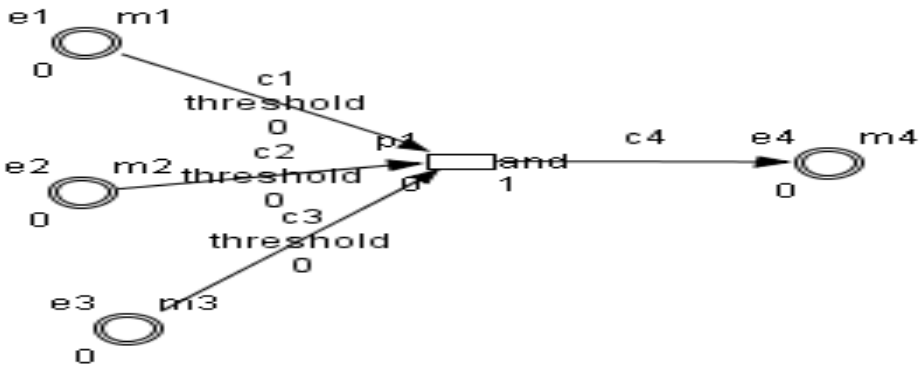


Figure 4.17 Hybrid Functional Petri Nets models of continuous elements.

Figure 4.18: Simulation of continuous entities and continuous process.

4.4.5 Initial Value and Threshold

For continuous entity, initial values are different from discrete entities. Values of continuous entities can be real number. For instance 0.6, 1.2, 1.8, …etc. Therefore values of discrete entities are only integer like 0, 1, 2, 3, 4, 5, …etc. Moreover, discrete entities' threshold value can be real number like continuous entity. Discrete entity and continuous entity threshold value's functions are same.

4.4.6 Speed and Delay

Continuous process is characterized by the speed parameter whereas discrete process is identified by the speed andthe delay parameters. Let us execute the model represented in Figure 4.19, and compare the result of simulation with the one in Figure 4.9.

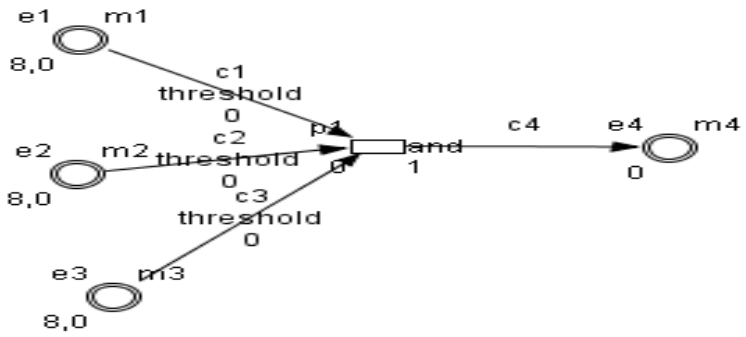


Figure 4.19 Modelling of continuous elements with speed and delay.

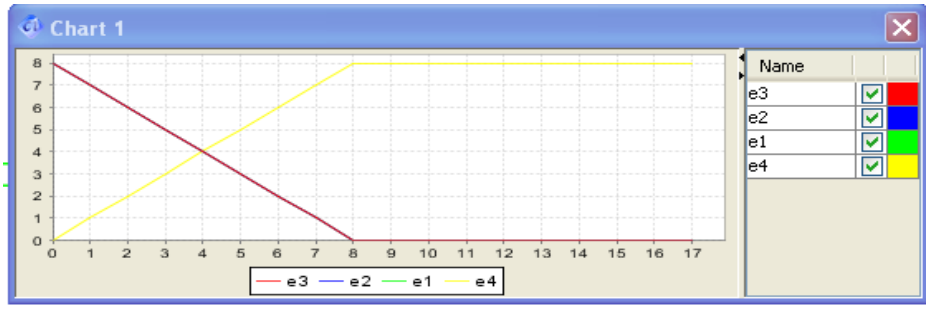


Figure 4.20 Simulationof continuous elements with speed and delay.

4.5 Pathway Models or Biological Pathways

In this section, we are going to be modeling the most known biological events.

4.5.1 Degradation

In the cell, mRNA and proteins are splitted naturally. This reaction is called degradation. These kinds of reaction can be modeling in three combinationsof the couple while using degradation process. These combinations of couples are (entity, process): (discrete, discrete), (continuous, continuous), and (continuous, discrete) like Figure 4.21. The simulation results are shown in Figure 4.22.

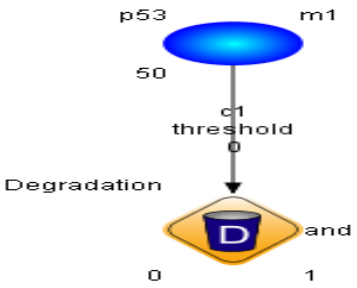


Figure 4.21Modeling of p53 and degradation.

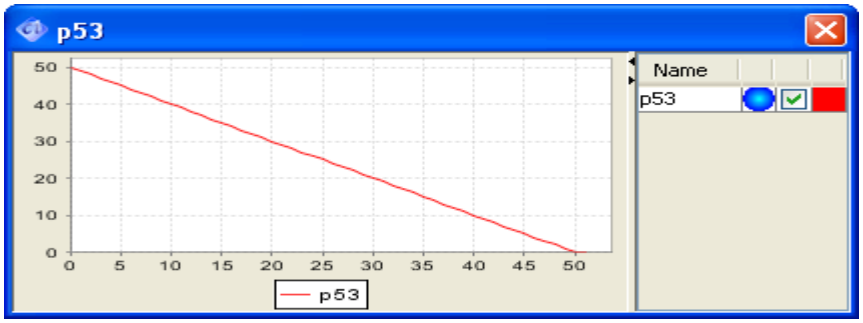


Figure 4.22 Simulation of p53 and degradation.

In above model, degration speed is not depended concentration of p53. To be more ralistic of degratation, dependency is added to p53 and also variable name(m1) is assigned to p53 then process parameter is changed to m1/10 using variable name(m1).

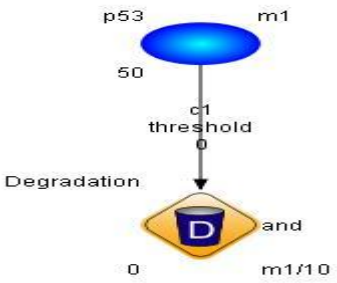


Figure 4.23Modeling of p53 and degradation with changed the process parameter.

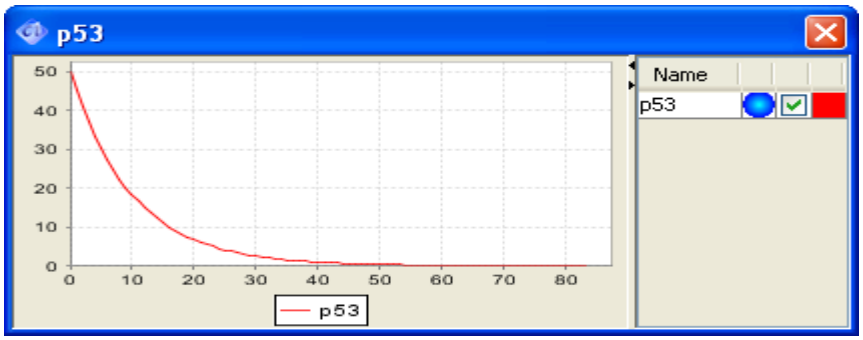


Figure 4.24Simulation of p53 and degradation with changed the process parameter.

4.5.2 Translocation

In the cells, mRNAs and proteins are carried to various places like from the (cytoplasm to the nucleus),or (from the nucleus to the Golgi body). This process is called translocation. Possible triples of the elements are (p53_nuclei, translocation, p53_cytoplasm): (discrete, discrete, discrete), (discrete, discrete, continuous), (continuous, discrete, discrete), (continuous, discrete,continuous) and (continuous, continuous,continuous) like shown in Figure 4.26. For model, there is only one combination, which is (continuous, continuous,continuous). Simulation results are as Figure 4.27.

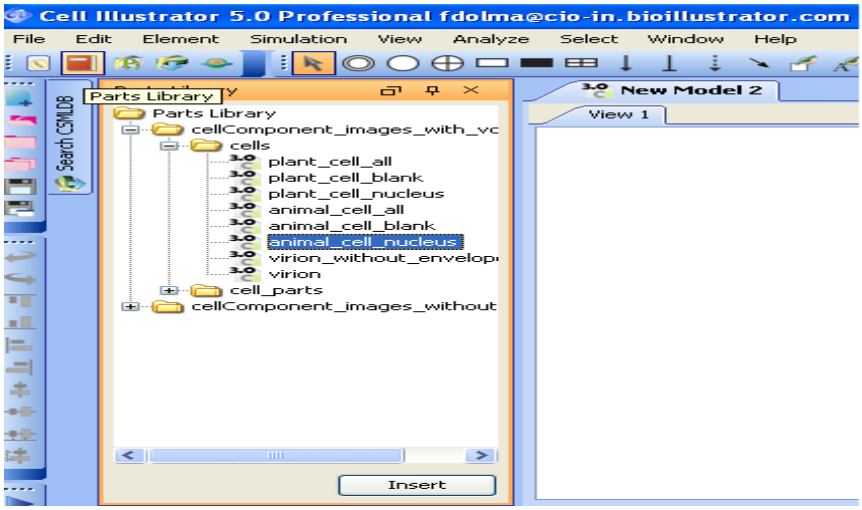


Figure 4.25: Parts library

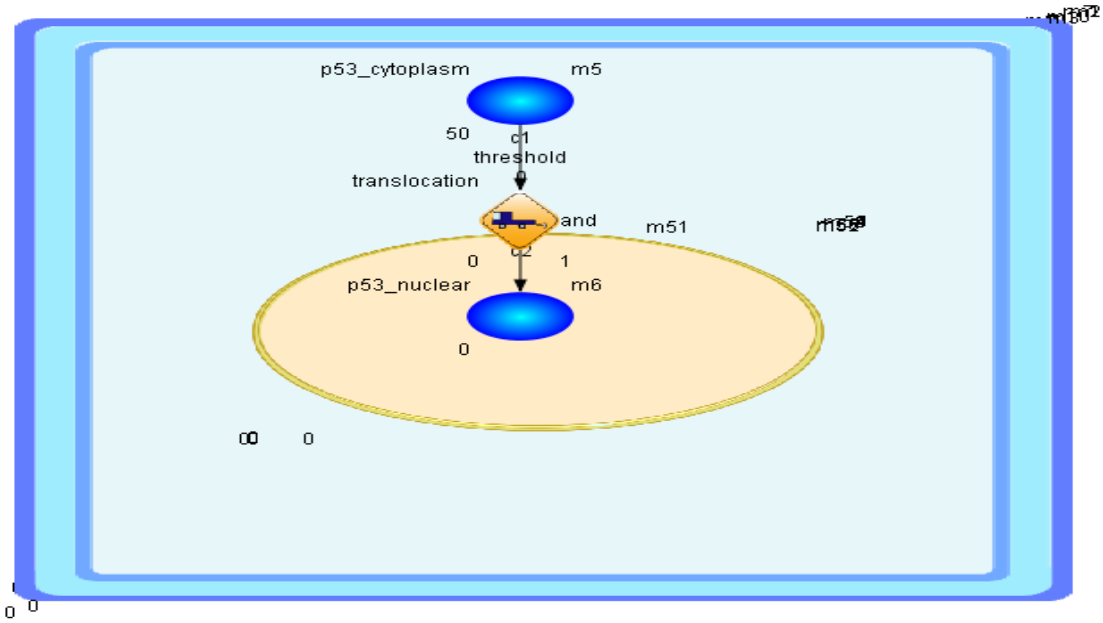


Figure 4.26: Modelling of p53_nuclei, translocation, p53_cytoplasm.

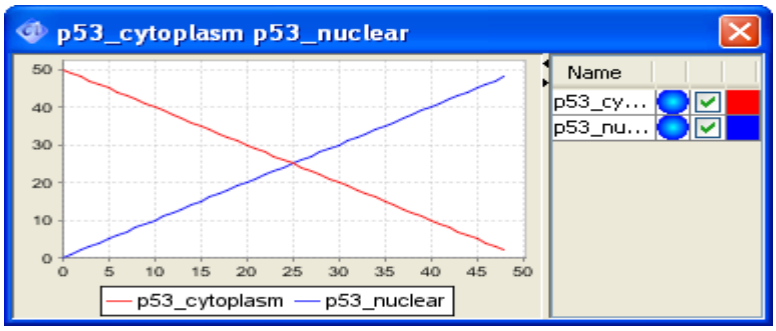


Figure 4.27: Simulation of p53_nuclei, translocation, p53_cytoplasm.

To connect Translocation speed to p53_nuclei entity (m5), process speed (m5) has to be a function. Example, $\frac{M5}{10}$ in Figure 4.28. More realistic model simulation is shown in Figure 4.29.

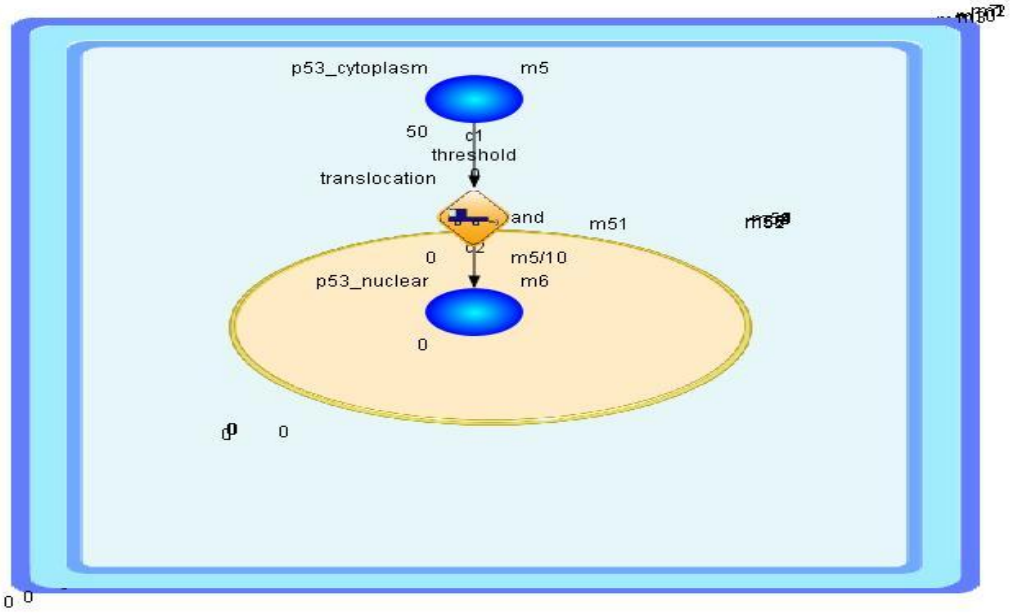


Figure 4.28HFPN model of p53_nuclei, translocation, p53_cytoplasm.

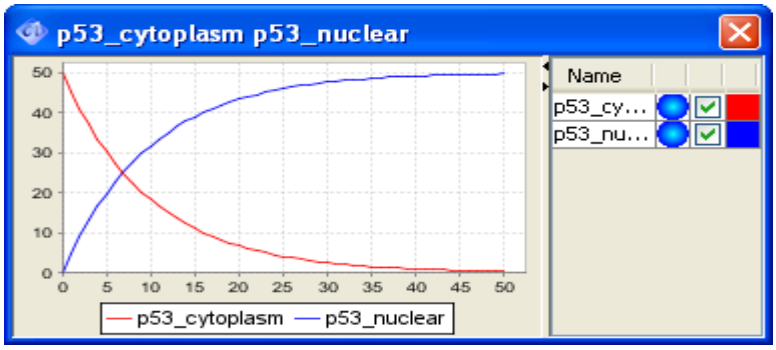


Figure 4.29 Simulation for result p53_nuclei, translocation, p53_cytoplasm.

4.5.3 Transcription

Using cell illustration, transcripton model can be perfomed easily. Model as Figure 4.30. For mRNA_p53 simulation figure as Figure 4.31. Possible combinations of elements are (transcription , mRNA_p53): (discrete, discrete), (discrete, continuous), (continuous, continuous).

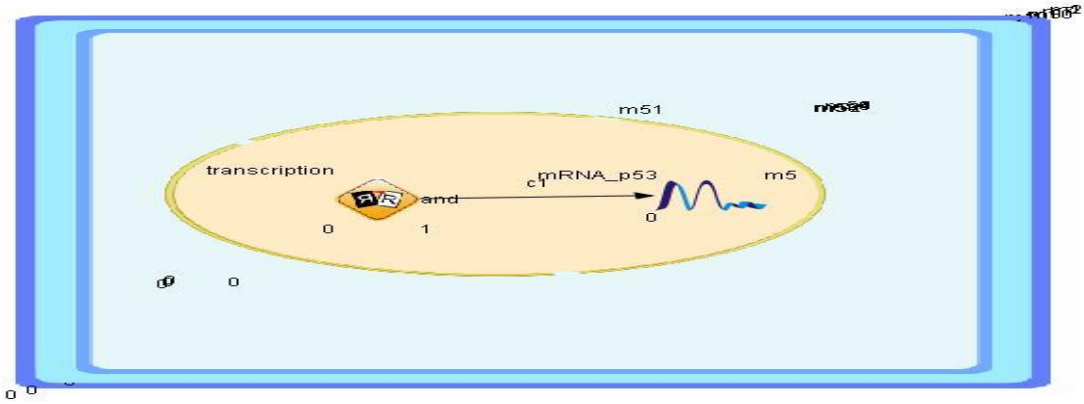


Figure 4.30 HFPN model oftranscription , mRNA_p53.

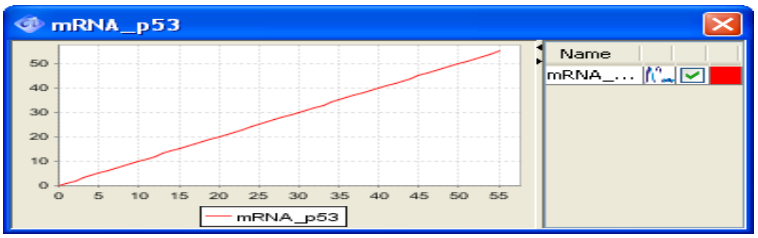


Figure 4.31Simulation for result transcription, mRNA_p53.

Normally mRNA is degrading at the same time as transcription. This model is shown down in Figure 4.32.Result of simulation is in Figure 4.33.

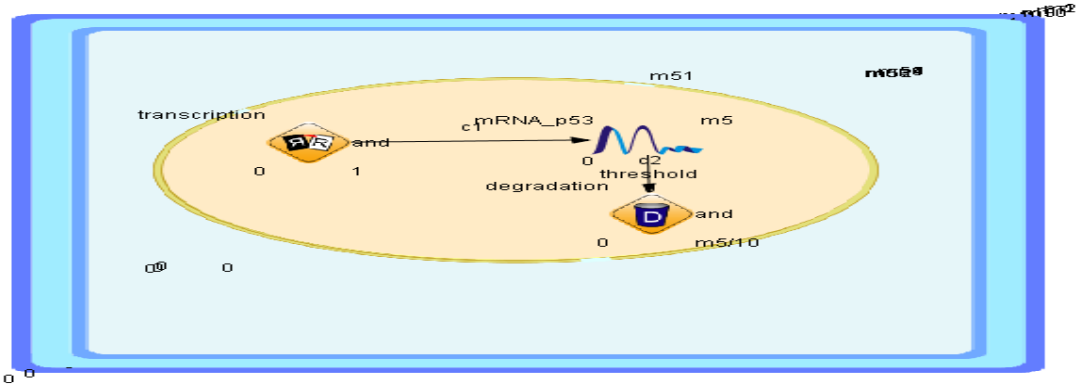


Figure 4.32 Model of transcription, mRNA and degradation.

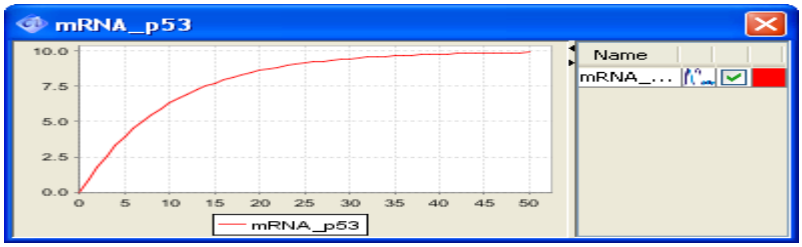


Figure 4.33Simulation result of transcription, mRNA and degradation.

4.5.4 Binding

In the cells, generally more than one proteins are combined to become a complex. This event is called binding. Binding process is modelled as Figure 4.34.

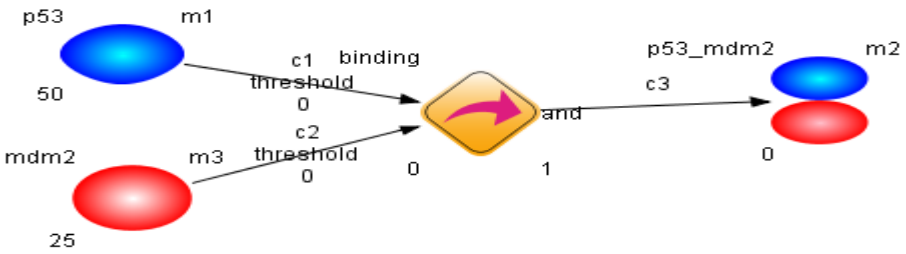


Figure 4.34 Model of binding

For all entities in this model one graphics has been constituted.Result in the Figure 4.35.

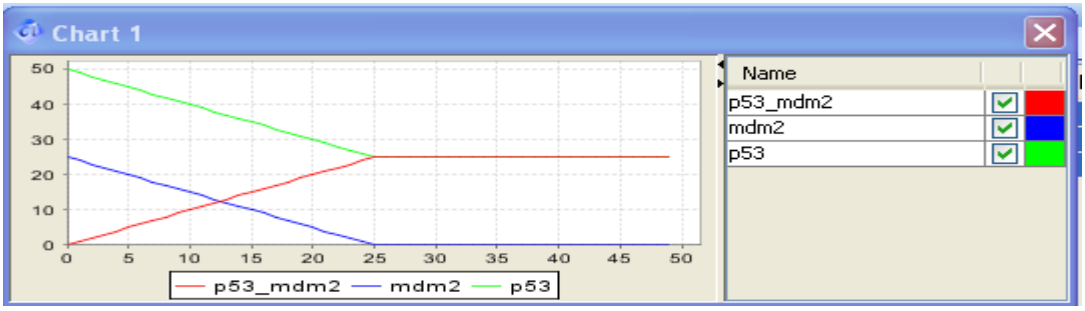


Figure 4.35 Simulation of binding

If binding rate comes over rate p53(variable m1) and mdm2(variable m2), process speed must be set. Setting is $\frac{m1 \times m2}{300}$(300 is arbitrary constant). Model is shown in Figure 4.36 and simulation is shown in Figure 4.37.

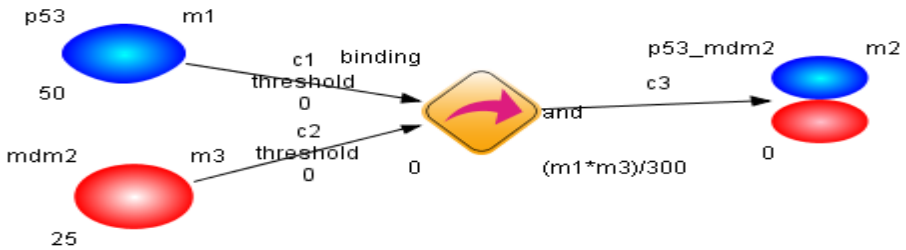


Figure 4.36Model of binding for change speed

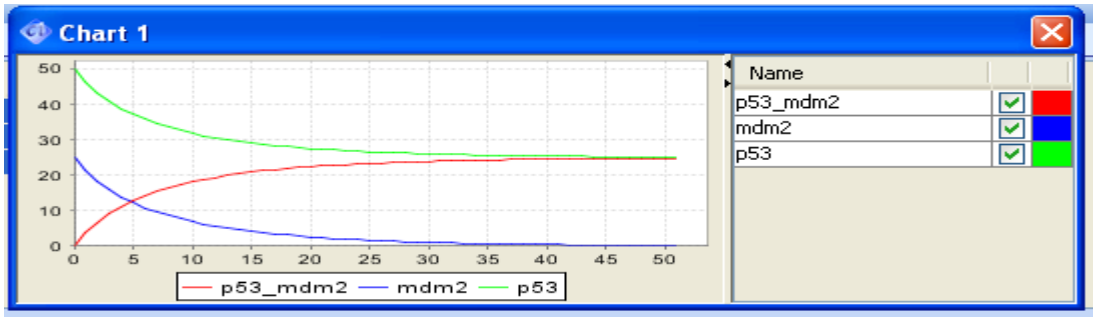


Figure 4.37 Simulationof binding for change speed

4.5.5 Inhibition

So far, models have been explanied using process connector. In this sample, inhibitor connector is going to be explained. Sometimes, inhibitor connector is used to inhibit special medicine for activation of transcription. Possible combination of elements are (process p1, doxorubicine): (discrete, discrete), (discrete, continuous), and (continuous, continuous). Only (discrete, discrete) combination is shown in Figure 4.38.

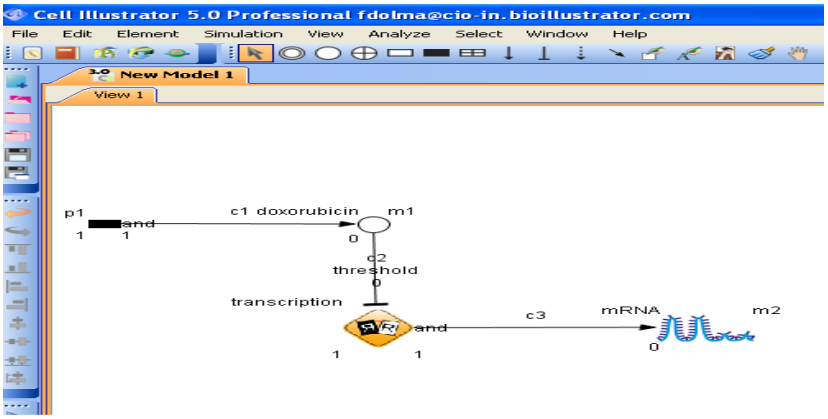


Figure 4.38 HFPN model ofprocess p1, doxorubicine

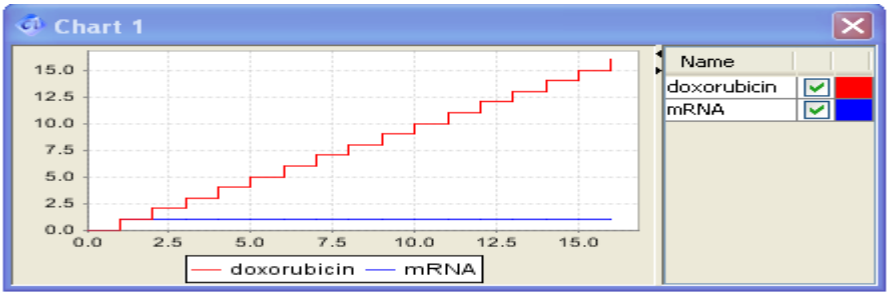


Figure 4.39 Simulation result for process p1, doxorubicine

An inhibitor connector model force (repression) connected process's response. Inhibitory connector depends on the relationship between the threshold rate of the connector, and the rate of the entity thus a process can be inhibited. If threshold rate of the connector smaller than rate of the entity then repression is enabled. In Figure4.38, doxorubicin is set to zero when the transcription progresses at the starting point. The transcription stops automatically when the value of doxorubicin becomes greater than zero. This is shown in Figure 4.39. The power of a drug is denoted by the threshold value of the inhibitory connector. When set the threshold value of the inhibitory connector to 5 as Figure 4.40.

The drug will be effective if the value of the entity representing the drug is above 5. If the value of the drug is under 5, the effect of the drug will be weak, as shown in Figure 4.41.

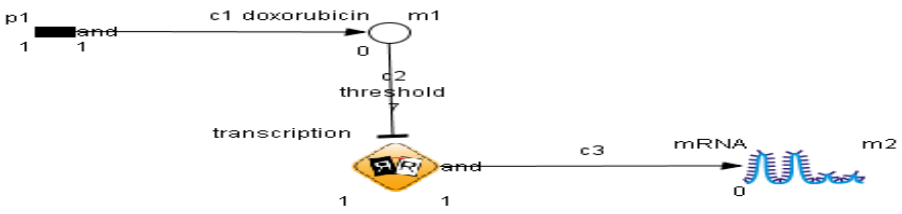


Figure 4.40HFPN model ofprocess p1, doxorubicine for change the threshold.

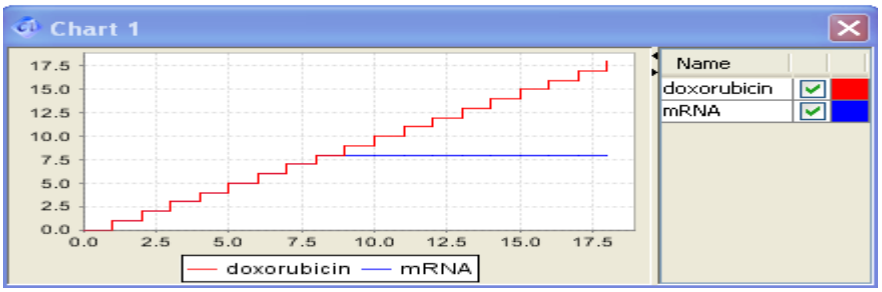


Figure 4.41Simulation result for process p1, doxorubicine for change the threshold.

Chapter 5

CASE STUDIES

In this chapter, we detail application of HFPNs in modelling and simulation of biochemical networks. Firstly we discuss the way HFPN can be used for modelling and simulation of signal transduction pathways, namely, interaction between MDM2, p19ARF and p53, to study transcriptional activity of p53. Then we exploit HFPN and Cell Illustrator software for modelling the regulatory mechanism for *lac* operon gene and glycolyticpathway in *E. coli*. Finally, we create HFPN model of circadian rhythm of *Drosophila*.

5.1Signal Transduction Pathway

5.1.1Validation of the p53 transcriptional activity through modelling and simulation with HFPN

In this subsection, we detail possible interactions between three proteins p53, MDM2 and p19ARF. p53 is a tumor suppressor protein that prevents cellsfrom dividing inappropriately.MDM2 is used as the name of a gene as well as the protein encoded by that gene. MDM2 is an important negative regulator of the p53 tumor suppressor. p19ARF is a tumor suppressor that interacts with p53 and MDM2.

In order to control transcriptional activity of protein p53, MDM2 and p19ARF come together and form a complex with protein p53. When p53 forms a functional dimer with protein MDM2, p53 loses its transcriptional activity. However, it can be

stillquestioned whether protein p53 continues its transcriptional activity when together with proteins MDM2 and p19ARF it forms a trimer.

The present examplewe gather information provided in the literature to create HFPN model and studythe interactionsbetweenp53, MDM2, p19ARF and their products. The simulation results clearly show the fact that protein p53 keeps on its transcriptional activity when p53, MDM2, and p19ARF form a trimer.

According to the information provided in the literature, possible interactions between p53, p19ARF and MDM2 can be categorized into 7 classes such as binding, transcription, translation, nuclear export, nuclear import, ubiquitination and degradation classes. Each class is characterized by multiplicity of the interaction types. Depending on components used there are 4 binding, 8 transcription, 3 translation, 1 nuclear export, 3 nuclear import, 1 ubiquitination and 1 degradation, a total of 21 different types of interactions between p53, p19ARF and MDM2 proteins. Biological data regarding interactions between p53, p19ARF and MDM2 are summarized in Table 5.1.

Table 5.1 Biological data characterizing interactions between p53, p19ARF and MDM2.

#	Biological phenomena	Action	Speed	Process
1	p53(N) is bound to MDM2(N)	T_1	$m_1 \cdot m_2 \cdot 0.01$	binding
2	MDM2(N) is bound to p19ARF	T_2	$m_2 \cdot m_4 \cdot 0.01$	binding
3	p53_MDM2(N) is bound to p19ARF(N)	T_3	$m_4 \cdot m_5 \cdot 0.01$	binding
4	MDM2_p19ARF is bound to p53(N)	T_4	$m_1 \cdot m_6 \cdot 0.01$	binding
5	Transcription of injected gene p53, producing p53 mRNA	T_5	1	transcription
6	p53 mRNA is translated to p53(N)	T_6	$m_{10} \cdot 0.1$	translation

7	p53_MDM2(N) is exported from the nucleus to the cytoplasm (p53_MDM2(C))	T_7	$m_5 \cdot 0.1$	nuclear export
8	p53 is marked with ubiquition (multiubiquition chain) (p53[Ub])	T_8	$m_7 \cdot m_8 \cdot 0.01$	ubiquitination
9	Polyubiquitinated p53 (p53[Ub]) is destroyedby proteasome	T_9	$m_9 \cdot 0.5$	degradation
10	Protein MDM2(C) is imported from the cytoplasm to the nucleus (MDM2(N))	T_{10}	$m_{12} \cdot 0.1$	nuclear import
11	Protein p53(C) is imported from the cytoplasm to the nucleus (p53(N))	T_{11}	$m_{11} \cdot 0.1$	nuclear import
12	Transcription of injected gene MDM2, producing MDM2 mRNA	T_{12}	1	transcription
13	MDM2 mRNA is translated to MDM2(C)	T_{13}	$m_{13} \cdot 0.1$	translation
14	Transcription of injected gene p19ARF, producing p19ARF mRNA	T_{14}	1	transcription
15	p19ARF mRNA is translated to p19ARF(C)	T_{15}	$m_{15} \cdot 0.1$	translation
16	Protein p19ARF(C) is imported from the cytoplasm to the nucleus (p19ARF(N))	T_{16}	$m_{16} \cdot 0.1$	nuclear import
17	Protein p53 (p53(N)) activates transcription of gene Bax, producing Bax mRNA	T_{17}	$m_1 \cdot 0.1$	transcription
18	Protein p53 (p53(N)) activates transcription of gene MDM2, producing MDM2 mRNA (endogeneous)	T_{18}	$m_1 \cdot 0.1$	transcription
19	Stabilizing p53 complex p53_MDM2_p19ARF activates transcription of gene Bax, producing Bax mRNA	T_{19}	$m_3 \cdot 0.1$	transcription
20	Stabilizing p53 complex p53_MDM2_p19ARF activates transcription of gene MDM2, producing MDM2 mRNA	T_{20}	$m_3 \cdot 0.1$	transcription
21	p19ARF could not affect to p53 transactivation without protein MDM2	-		transcription

Genomic Object Net software tool has been used in [34] for modelling of biopathways and conducting similar experiments. We have used a new version of Genomic Object Net, which is called Cell Illustrator 5.0, for creating HFPN model of biopathway and performing the simulations. A Cell Illustrator screen snapshot with HFPN model of interactions between p53, MDM2 and p19ARF on it is illustrated in Figure 5.1.

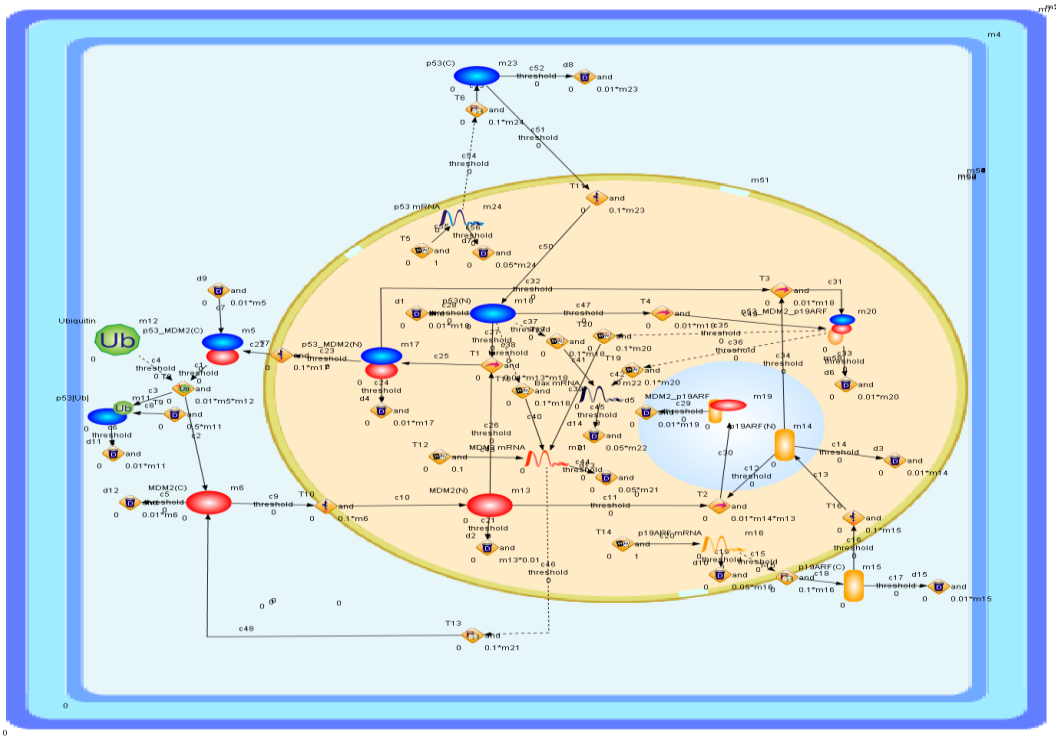


Figure 5.1 HFPN model of interactions between p53, MDM2 and p19ARF.

Another snapshot of the Cell Illustrator screen, which illustrates simulation results of concentration behaviors of p53, MDM2, p19ARF and p53_MDM2_p19ARF, is shown in Figure 5.2.

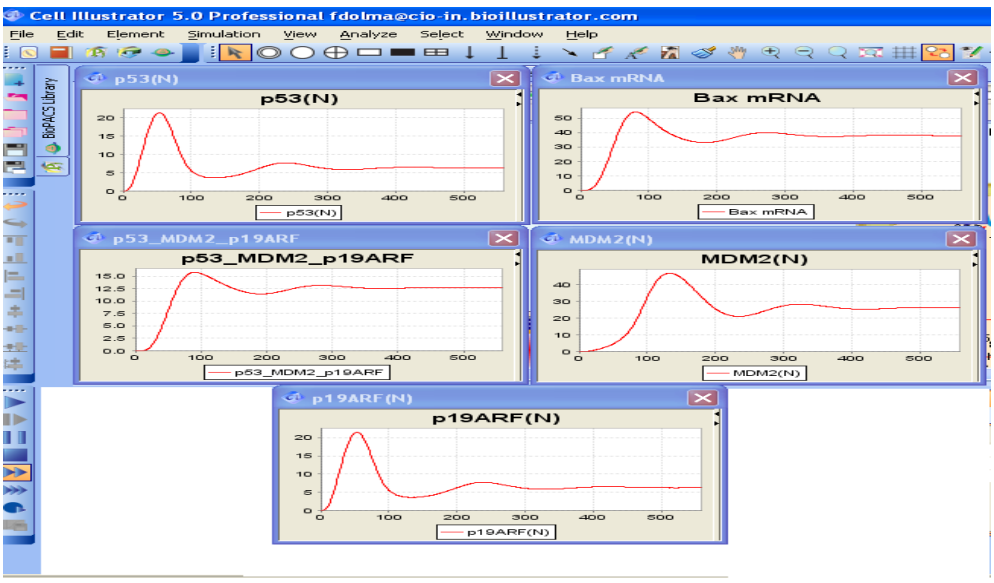


Figure 5.2Simulation results of concentration behaviors of p53, MDM2, p19ARF and p53_MDM2_p19ARF.

5.2Metabolic Pathway

5.2.1 Modelling of the *lac* operon gene regulatory mechanism and glycolytic pathway in *E. coli*with HFPN

In this section,we exploit HFPNs to create an HFPN model of the gene regulatory mechanism for *lac* operon and glycolyticpathway in *E. coli*. It must be noticed that discrete Petri net for modelling glycolytic pathways was first considered by in [109]. Since that time colored Petri nets [136], stochastic Petri nets [40] and HFPNs [32] have successfully implemented for creating adequate models. In the present case study we use HFPNs, that combine both discrete and continous features, to modelling glycolytic pathway and *lac* operon in *E. coli*. HFPN model is created according to the known biological knowedge that is gathered from many sources [32]. All the parameters in the transitions of the HFPN model are summarized in Table5.2.

Table 5.2 Transitions and related parameters in HFPN model.

v_1	v_2	v_3	From			To		Comment
			v_4	v_5	v_2	v_4	v_5	
T_1	C	m_4/10	m_4	0	N	-	-	degradation rate of CAP
T_2	C	m_{14}/10	m_{14}	0	N	-	-	degradation rate of mRNA repressor
T_3	C	m_{15}/10	m_{15}	0	N	-	-	degradation rate of repressor
T_4	C	m_{17}/10	m_{17}	0	N	-	-	degradation rate of repressor binding to DNA
T_5	C	m_{18}/10	m_{18}	0	N	-	-	degradation rate of repressor not binding to DNA
T_6	C	m_7/10	m_7	0	N	-	-	degradation rate of repressor binding to operator region
T_7	C	m_{19}/10	m_{19}	0	N	-	-	degradation rate of *lacZ* mRNA
T_8	C	m_{20}/10000	m_{20}	0	N	-	-	degradation rate of *lacZ*
T_9	C	m_{23}/10	m_{23}	0	N	-	-	degradation rate of *lacY* mRNA
T_{10}	C	m_{24}/10	m_{24}	0	N	-	-	degradation rate of *lacY*
T_{11}	C	m_{27}/10	m_{27}	0	N	-	-	degradation rate of *lacA* mRNA
T_{12}	C	m_{28}/10000	m_{28}	0	N	-	-	degradation rate of *lacA*

T_{13}	C	m_{29}/10000	m_{29}	0	N	-	-	degradation rate of lactose outside of cell of lactose outside of cell
T_{14}	C	m_9/10000	m_9	0	N	-	-	degradation rate of lactose
T_{15}	C	m_8/2	m_8	0	N	-	-	degradation rate of arolactose
T_{16}	C	m_{30}/10000	m_{30}	0	N	-	-	degradation rate of galactose
T_{17}	C	m_{17}/10000	m_{17}	0	N	-	-	degradation rate of glucose
T_{18}	C	m_{10}/10000	m_{10}	0	N	-	-	degradation rate of complex
T_{19}	C	m_5/10000	m_5	0	N	-	-	degradation rate of cAMP
T_{20}	C	m_{11}/10000	m_{11}	0	N	-	-	degradation rate of AMP
T_{21}	C	m_{12}/10	m_{12}	0	N	-	-	degradation rate of ADP
T_{42}	D	1	m_2	1	N	-	-	CAP releasing rate
T_{43}	D	1	m_3	1	N	-	-	repressor releasing rate
T_{45}	C	1	-	-	N	m_4	-	CAP production rate
T_{46}	D	1	-	-	-	m_{13}	1	activation of repressor gene
T_{57}	D	1.082	m_{13}	1	N	m_{14}	1	transcription rate of repressor
T_{58}	C	m_{14}	m_{14}	0	N	m_{15}	-	transcription rate of repressor mRNA
T_{59}	C	-	m_{15}	-	C	m_{16}	-	confirmation rate of repressor
T_{60}	C	$96 \times m_{16}/100$	m_{16}	0	N	m_7	-	repressor binding rate to operator
T_{61}	C	$399 \times m_{16}/10000$	m_{16}	0	N	m_{17}	-	repressor binding rate to the DNA other than repressor site
T_{62}	C	$m_{16}/1000$	m_{16}	0	N	m_{18}	-	rate of repressor which does not bind any DNA
T_{63}	D	1	m_4	1	T	m_2	1	binding rate of CAP to the CAP site
T_{64}	D	1	m_5, m_7	100,1	T,T	m_3	1	binding rate of repressor to the operon
T_{65}	D	1	m_2, m_8	4,1	I,T	m_1	1	logical operation of the places “CAP site” and “operator”
T_{66}	D	3.075	m_3, m_1	1, 1	I, T	m_{19}	1	transcription rate of *lacZ*
T_{67}	C	m_{19}	m_{19}	1	T	m_{20}	-	translation rate of *lacZ*
T_{68}	D	0.051	m_{21}	1	N	m_{22}	1	moving rate of RNA polymerase
T_{69}	D	1.254	m_{22}	1	N	m_{22}, m_{25}	1, 1	transcription rate of *lacY*
T_{70}	C	m_{23}/2	m_{23}	1	T	m_{24}	-	transcription rate of *lacY*
T_{71}	D	0.065	m_{25}	1	N	m_{26}	1	moving rate of RNA polymerase
T_{72}	D	0.682	m_{26}	1	N	m_{27}	1	transcription rate of *lacA*
T_{73}	C	m_{27}/5	m_{27}	1	T	m_{28}	-	translation rate of *lacA*
T_{74}	C	$\frac{m_{24} \times m_{29}}{m_{29} + m_{24} \times 10}$	m_{24},m_{29}	2.5,0	T,N	m_{30}	-	
T_{75}	C	$\frac{m_{20} \times m_9}{m_9 + m_{20} \times 10}$	m_{20}, m_9	5	T	m_6	-	Decomposition rate of lactose to galactose and glucose
T_{76}	C	m_9/5	m_9	1	T	m_8	-	producing rate of allolactose from lactose inside of a cell

T_{77}	D	0.5	m_3, m_8	1, 1	N,N			confirmation rate of repressor and all llolactose
T_{79}	C	m_5/10	m_5	0	N	m_{11}	-	reaction rate: cAMP to AMP
T_{80}	C	m_{11}/10	m_{11},m_6	0,5	N,I	m_5	-	reaction rate: AMP to cAMP
T_{81}	C	m_{11}/10	m_{11}	0	N	m_{12}	-	reaction rate: AMP to ADP
T_{82}	C	m_{12}/10	m_{12}	0	N	m_{11}	-	reaction rate: ADP to AMP
T_{84}	C	m_{29}/10	m_{29}	0	T	m_8	-	producing rate allolactose from lactose outside of a cell

In the above tablev_1,v_2, v_3,v_4 and $v_5$stand for name, type, delay/speed, variable and weight, respectively.

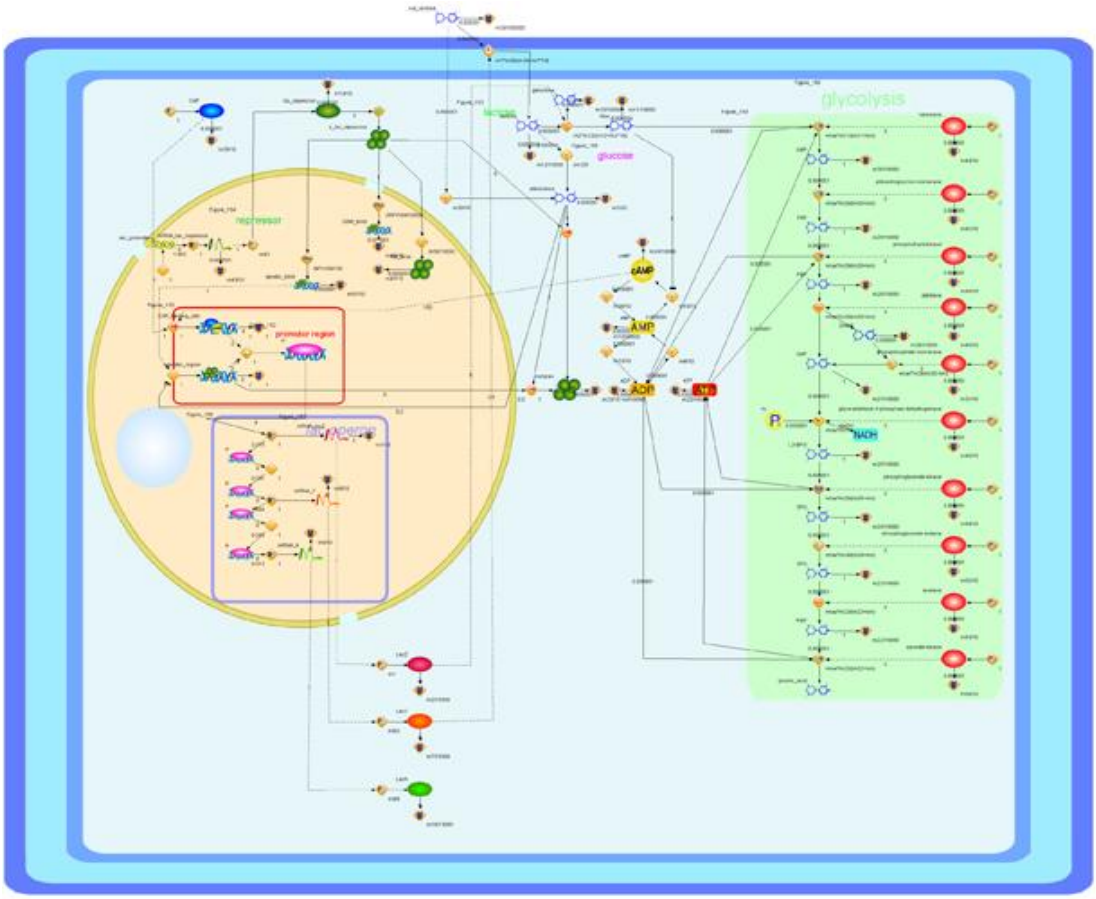


Figure 5.3 HFPN model of*lac* operon gene regulatory mechanism of *E. coli*.

Together with modeling of the glycolytic pathway, we model the *lac* operon gene regulatory mechanism of *E. coli* with HFPN, which includes the dual control of the

lac operon participating *lac* repressor, allolactose, catabolite gene activator protein (CAP), and cyclic AMP (cAMP). The modeling is simply done by mapping the information represented in Figure 5.4 and Figure 5.5and Figure 5.6 to places and transitions. HFPN model of *lac* operon gene regulatory mechanism and glycolytic pathway in *E. coli*is created in Cell Illustrator software and is shown in Figure 5.3.

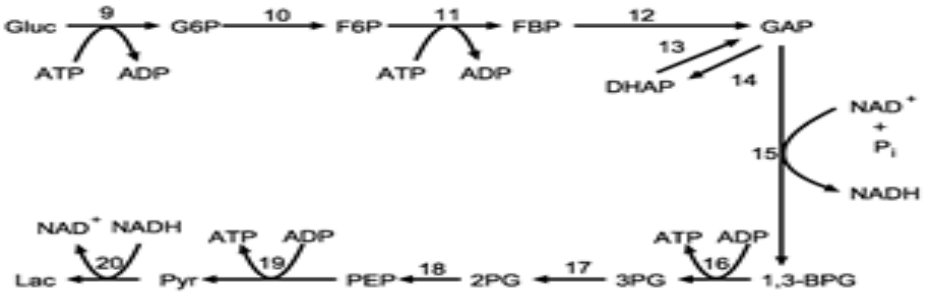


Figure 5.4 A part of the glycolytic pathway.

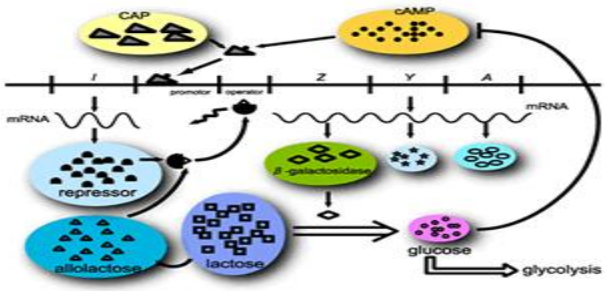


Figure 5.5 LacZ, the first gene of the *lac* operon, encodes the enzyme b-galactosidase which breaks down lactose to galactose and glucose.

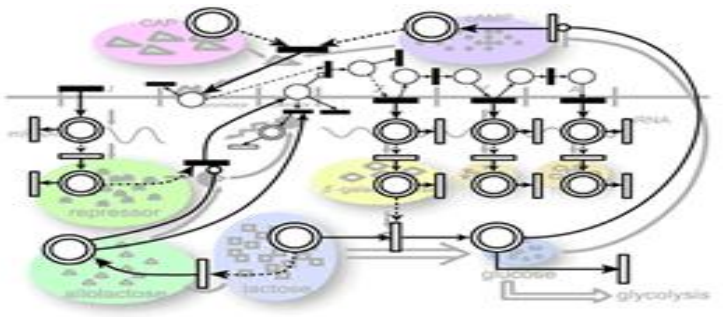


Figure 5.6 HFPN model of the regulatory mechanism.

It took 22.5 sec with Cell Illustrator 5.0 to get the complete simulation results for HFPN model of*lac* operon gene regulatory mechanism of *E. coli*. Simulation results are illustrated in Figure 5.7.

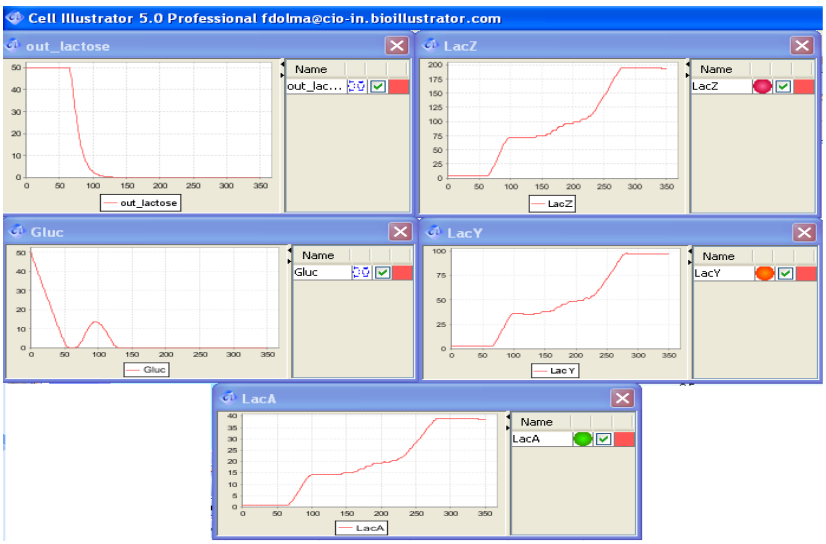


Figure 5.7: Simulation results for HFPN model of*lac* operon gene regulatory mechanism of *E. coli*.

5.3 Genetic Regulatory Network

We have chosen circadian rhythms in *Drosophila* as an example to illustrate use of HFPNs and Cell Illustrator for modelling genetic regulatory networks. Five genes period (per), timeless (tim), Drosophila clock (dClk), cycle (cyc) and double-time (dbt) participate in control mechanism of autoregulatory feedback loops of *Drosophila melanogaster*.

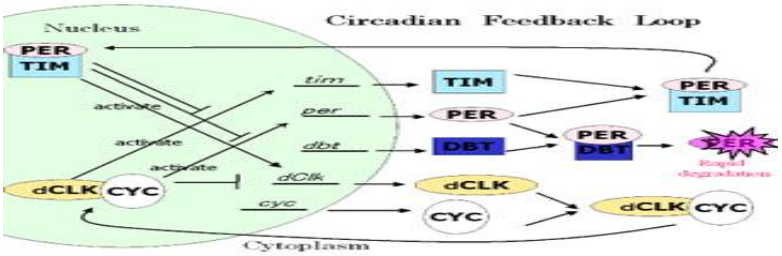


Figure 5.8: Representation of the gene regulation mechanism in the *Drosophila* circadian rhythm.

I must be noticed that PER and TIM proteins collaborate in the regulation of their expression in *Drosophila*, building in PER-TIM complexes that permit nuclear translocation, inactivation of *per* and*tim* transcription in a cycling negative feedback loop, and activation of dClk transcription which participates in the dCLK-CYC negative feedback loop. The dCLK and the CYC form heterodimers that activate *per* and *tim* transcriptions and inhibit dClk transcription.

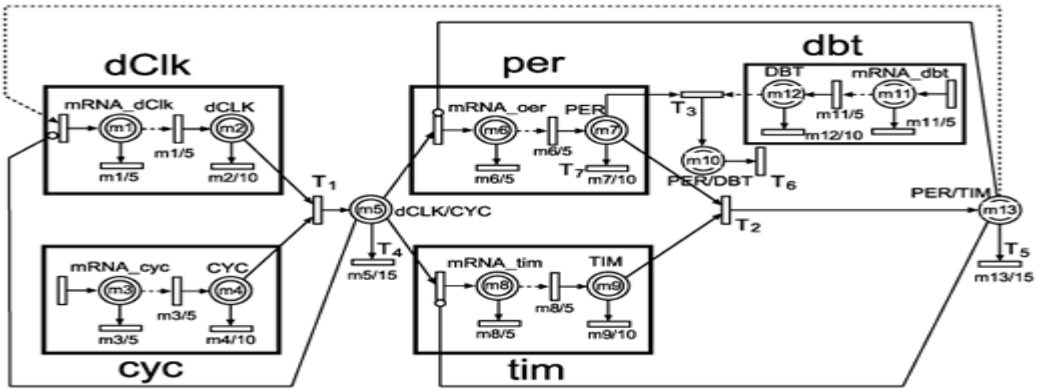


Figure 5.9: A representation of *Drosophila* circadian mechanism involving the genes *per*, *tim*, *dClk*, *cyc*, and *dbt* .

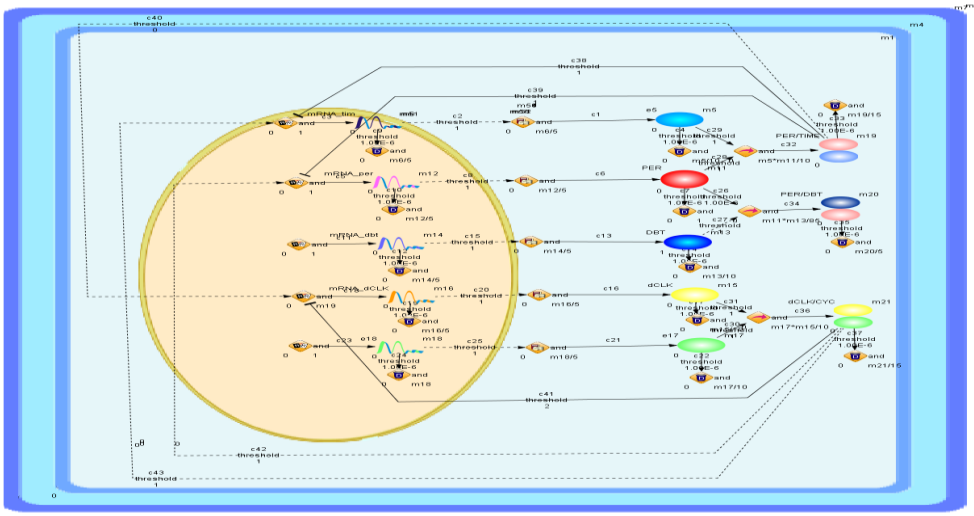


Figure 5.10: A HFPN representation of *Drosophila* circadian mechanism.

We gather information provided in Figure 5.8 and Figure 5.9 and create a HFPN model in Cell Illustrator. In this model, places and transitions are chosen accordingly and the increase/decrease rate in amount of proteins and complexes is

$\frac{m_{11}\times m_{10}}{20}(\frac{m4\times m5}{20}, \frac{m4\times m7}{20})$ for the proteins dCLK (m11) and CYC (m10) (the proteins PER (m4) and TIM (m5), the proteins PER (m4) and DBT(m7)). This rate is assigned to transition T1 (T2, T3). Transitions T4, T5, and T6 represent the degradation rates of complexes of the corresponding proteins. Related model is shown in Figure 5.10. Simulation results for Drosophila circadian mechanism are depicted in Figure 5.11.

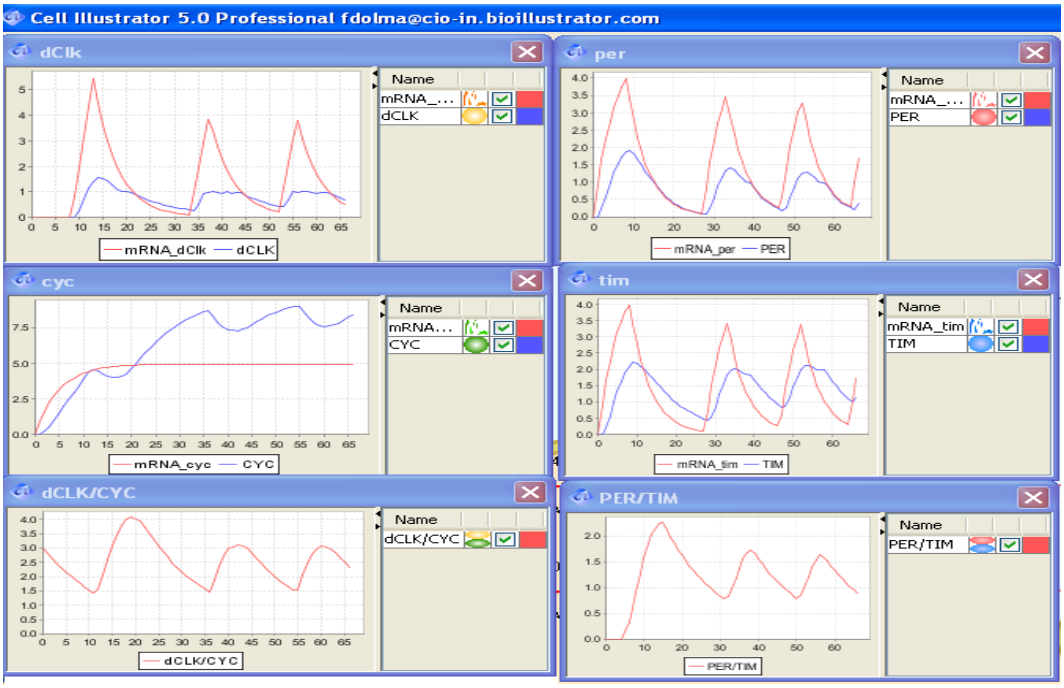


Figure 5.11: Simulation results for Drosophila circadian mechanism.

CONCLUSIONS

It is the aim of the present thesis to study interaction between Petri nets and biological sciences, to the benefit of both fields. On the onehand we observe that there is growing demand in application of Petri nets in biochemistry, biomedicine, molecular biology and systems biology, which consequently widens the spectrum of their application areas;on the other hand, Petri net involved modelling and simulation allows researchers to get broader view of biological processes and dive deep into the details and understand nature of biological systems.

The main outcomes of this thesis are summarized as follows: (1) a systematical study of bibliography on application of Petri nets in biosciences that covers the period of January 1993 to July 2010; (2) study of up-to-date and powerful Petri net based research methods and skills on example of validation of the p53 transcriptional activity through simulating its HFPN model in Cell Illustrator, modelling of the *lac* operon gene regulatory mechanism and glycolytic pathway in *E. coli*, and modelling and simulation of circadian rhythms in *Drosophila*.

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